Int. J. Bio-Inorg. Hybr. Nanomater., 10(1): 43-51, Spring 2021

Loop-Mediated Isothermal Amplification assay for rapid detection of G1 human *Rotavirus*

M. Kargar[1], N. Hoveizeh Tamimian[2*], A. Doosti[3], F. Moghadamdizajherik[4], F. Mazaheri-Eftekhar[5]

[1] Department of Microbiology, Jahrom Branch, Islamic Azad University, Jahrom, Iran

[2] Department of Biology, Islamic Azad University Tehran North Branch, Tehran, Iran

[3] Biotechnology Research Center, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

[4] Department of Immunology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

[5] Department of Nutrition, Science and Research Branch, Islamic Azad University, Tehran, Iran

Received: 14 October 2020; Accepted: 16 December 2020

ABSTRACT: We developed a Loop-Mediated Isothermal Amplification (LAMP) assay for rapid detection of the most prevalent Rotavirus genotype (G1) responsible for the hospitalization of children worldwide, and compared the sensitivity of LAMP with nested-PCR. A total of 365 stool samples from young children were analyzed by using 6 set of primers targeting conserved sequences of VP7 gene, within 90 min, under isothermal conditions at 62°C, by only a regular laboratory heat block. Then, the LAMP products were analyzed by using gel electrophoresis and the naked-eye after adding SYBR Green I. A ladder pattern on gel electrophoresis was observed specifically only for G1 Rotaviruses and not for other viruses. This LAMP reaction had the same sensitivity as a nested-PCR assay, the detection limit for the both systems were found to be 10 copies ml^{-1} of G1 Rotavirus RNA. The LAMP assay reported here is faster than nested PCR, cost-effective, and easy to perform and will be valuable tool for rapid and reliable clinical diagnosis of *Rotavirus* in developing countries.

Keywords: *Isothermal, LAMP, Nested PCR, Rotavirus, VP7 gene*

INTRODUCTION

Diarrheal diseases are still a major health problem throughout the world and are associated with considerable morbidity and a substantial number of hospitalizations among children [1]. Each year, about 111 million episodes of gastroenteritis due to *rotavirus* are reported in children worldwide of which 2 million require hospitalizations and 400,000 deaths occur [2]. *Rotavirus* is member of the Reoviridae Family, and classified into seven groups (A-G) [3]; Group A *Rotavirus* is the most important cause of severe gastroenteritis worldwide and The RVA outer capsid proteins VP7 and VP4 contain neutralization antigens, and by neutralization assays, have been classified into G and P serotypes, respectively [4]. Apart from these serotypes, G and P genotypes based on genetic diversity of the VP7 and VP4 genes, respectively, have been defined, and at least 27 G types and 37 P types have been discriminated so

(*) Corresponding Author - e-mail: neda.tamimian@gmail.com

far [4-6]. Studies of the genotyping in different countries have been indicated that G1 *Rotavirus* (RVA-G1) is the most prevalent genotype, and it has been detected in frequencies ranging from 36% to 74% in different regions of the world [7,8]; The clinical symptoms associated with RV gastroenteritis are not sufficiently characteristic to distinguish between RV infection and other causes of gastroenteritis [9]. Therefore, it is important to establish a sensitive, simple, cost-effective, and rapid diagnostic techniques for detection of RVA-G1, because children with testing positive for *Rotavirus* do not require antibiotic treatment unless warranted by a bacterial infection. The most frequently used methods for the detection of *Rotavirus*es are electron microscopy (EM), Enzyme-linked immunosorbent assay (ELISA), and reverse transcription-polymerase chain reaction (RT-PCR) [7]; Examination of RV samples by EM reveals the characteristic morphology of RV particles. However, the sensitivity of this technique is low because of poor conditions of sample transport to the laboratory and the method is too labor intensive for routine detection of *Rotavirus* in large numbers of stool specimens [10-12]. The ELISA and PCR assays are highly sensitive and specific [13], but in antibody-based assays, many factors such as antigenic drift and cross-reactivity may cause false positive and negative results [14,15]. Moreover, the requirement for a high precision thermal cycler in PCR prevents its powerful method from being widely used [16]. In 2000, Notomi *et al.* reported a novel nucleic acid amplification method, termed the loop-mediated isothermal amplification (LAMP) that relies on autocycling strand displacement DNA synthesis performed by using the *Bst* DNA polymerase large fragment [17]. The LAMP reaction typically amplifies target sequences with high specificity and efficiency under isothermal conditions, and can be conducted with a simple heating block. Thus, the thermal-cycling needs of a PCR are avoided [18,19]. Also, the LAMP assay produces a large amount of amplified products, resulting in easier detection by visual inspection, by observation of colour change after addition of SYBR green I dye [20]. The alternative product detection system using ethidium bromide has several limitations, such as generation of hazardous waste and sensitivity that is 25–100 times less than that of SYBR Green I [20]. Therefore, the LAMP assay has the advantage in specificity, sensitivity and rapidity over other nucleic acid amplification methods and could potentially be a valuable tool for the rapid diagnosis of infectious diseases in both clinical and hospital laboratories of developing countries [21]. The aim of this study was to develop the LAMP method for detection of RVA-G1 and compared its sensitivity to nested-PCR.

MATERIALS AND METHODS

Clinical samples

A total of 365 stool samples were obtained from children (aged under 5 years old) who were hospitalized with acute gastroenteritis symptoms in several hospitals of different regions of the South of Iran, from November 2009 to October 2010. All of the samples were collected 1 to 3 days after disease onset and stored at -70°C until further investigation. Then, RVAs were immunologically diagnosed in stool samples by a solid-phase sandwich-type enzyme immunoassay method (*Rotavirus* Ag ELISA, DRG, Germany) according to the manufacturer's instructions and RVA-positive samples were subjected to nested-PCR and LAMP reactions. One RVA-G1 sample for which the copy numbers of viral RNA had previously been determined by the quantitative genesig kit (Promega) was used as positive control for the design and analysis of sensitivity of LAMP assay.

RNA extraction and cDNA synthesis

Genomic RNA was extracted directly from stool samples by using RNXTM (plus) buffer (CinaGen Co., Tehran, Iran) and following the manufacturer's protocol. Complementary DNA (cDNA) was synthesized from 5 µL of eluted RNA using random hexamer primers and the Revert Aid First Strand cDNA kit (K1621; Fermentas, Germany) following the manufacturer's instructions. The cDNA product was subjected to nested-PCR and LAMP.

Nested multiplex PCR for G genotyping

Nested multiplex PCR was performed for G genotyping using consensus and type-specific primers [22]. The location and sequences of the primers are given

in Table 1. In brief, the PCR reaction was carried out in a total volume of 50 µl containing 10X PCR buffer, MgCl2 (50 mM), dNTPs (10 mM), 1.5 U *Smar* Taq DNA polymerase (CinaGen Co., Tehran, Iran), 10 µl of viral cDNA and the consensus primers Beg9 and End9 (5 pM), to amplify a 1062-bp fragment. In the second round of PCR, 5 µl of undiluted first-round PCR product was added to multiplex reaction mix similar to the first but using G type-specific primers, aBT1 (G1), aCT2 (G2), aET3 (G3), aDT4 (G4), aAT8 (G8) and aFT9 (G9), mG10 (G10) and G12. The cycle conditions were identical for both rounds, and were: denaturation at 94°C for 1 min; 30 cycles (for second run, 20 cycles) of amplification at 42°C for 2 min, 72°C for 2 min and a final extension at 72°C for 5 min. The reactions were carried out in a Mastercycler Gradient Thermocycler (Eppendorf, Hamburg, Germany). PCR products were visualized using 1.2 % ethidium bromide stained agarose gel electrophoresis. RVA-G1 specimens were used for LAMP reaction.

Primer design for LAMP reaction

The oligonucleotide primers used for LAMP reaction were designed from the N terminus region of VP7 gene sequences of *Rotavirus* G1 strain DL7 obtained from GenBank (Accession No: GU985239.1). First, the VP7 gene sequences from G1 *Rotavirus*es were obtained from Gen- Bank and aligned by the Mega 4 software (http://www.megasoft ware.net) to identify conserved regions. From the aligned sequences, the potential target region of the 228-bp corresponding to genome positions 778-1006 was selected, and LAMP primers were designed by the GeneRunner software (http://www.generunner.net/). A set of six primers containing two outer (B3, F3), two inner (FIP, BIP) and two loop primers were designed.

LAMP reaction

The LAMP reaction was performed in a total volume of 25 µl reaction mixture containing 1.6 µM each of FIP and BIP, 0.2 µM each of F3 and B3, 0.4 µM each of loop-F and loop-B, 2.5 µl Thermopol buffer 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 9 mM $MgSO_4$ and 0.1 % Triton X-100), 1.4 mM deoxynucleoside triphosphate mix, 1.0 M Betain (Sigma-Aldrich, St. Louis, MO, USA), 5 µl of target cDNA, and distilled water. The mixtures were heated to 95°C for 5 min, and then chilled on ice prior to addition of 8 U of *Bst* DNA polymerase large fragments (New England Biolabs). Immediately after addition of the polymerase, the mixture was incubated at 62°C for 90 min in a conventional heating block (SBH 130D; Stuart Scientific, Staffordshire, UK) and then heated at 80°C for 10 min to terminate the reaction.

Analysis of LAMP product

LAMP products were subjected to 3% agarose gel electrophoresis and visualized under UV light after ethidium bromide staining; a 100-bp DNA Ladder marker (Fermentas, Genruler, Germany) was used to determine the size of the products. In addition, the products were detected visually by a colour change following the addition of 10 µL of SYBR Green I (Invitrogen lot: 49743A) diluted 1:100 to tubes containing LAMP products. The solution turned green if LAMP reaction products were present, otherwise it remained orange; colouration was evaluated under natural light and UV light (302 nm; via handheld UV torch lamp).

Specificity and sensitivity of the LAMP reaction

The specificity of the LAMP primers for the VP7 gene of RVA-G1 was first checked by comparing the primer sequences to the GenBank database using BLAST software (available at http://www.ncbi.nih.nlm.Gov). The specificity of the LAMP primers was experimentally established by checking the cross-reactivity with genome of other known enteric viral pathogens including adenovirus group F, *norwalk virus*, sapovirus and astrovirus. To test the ability of the LAMP reaction to detect low concentrations of RVA-G1 in stools, we performed a 10-fold serial dilution of a positive control RNA which containing 10^4 copies ml^{-1}, from 10^4 to 10^0. Each dilution was used for LAMP and RVA-G1 nested-PCR assays.

RESULT

Rotavirus genotyping and nested PCR result

RVA was confirmed in 154 of 365 (42.2 %) stool samples analyzed by ELISA methods. Then, G genotyping was performed on these positive samples by using

Table 1. Details of type-specific nested-PCR and LAMP primers for detection of Rotavirus genomic RNA

	Primer	Sequence (5'→3')	Position	Genotype
nested-PCR primers	Beg 9	GGC TTT AAA AGA GAG AAT TTC CGTCTG G	1-28	G
	End 9	GGT CAC ATC ATA CAA TTC TAA TCT AAG	1062-1036	G
	aBT1	CAA GTA CTC AAA TCA ATG ATG G	314-335	G1
	aCT2	CAA TGA TAT TAA CAC ATT TTC TGT G	411-435	G2
	aET3	CGT TTG AAG AAG TTG CAA CAG	689-709	G3
	aDT4	CGT TTC TGG TGA GGA GTT G	480-498	G4
	aAT8	GTC ACA CCA TTT GTA AAT TCG	178-198	G8
	aFT9	CTA GAT GTA ACT ACA ACT AC	757-776	G9
	G10 or mG10	ATG TCA GAC TAC ARA TAC TGG	666-687	G10
	G12	CCG ATG GACGTAACGTTGTA	548 - 567	G12
LAMP primer	F3	TGTACTATTCGAAATTGTAAGAAGTT	778-803	
	B3	CGGAATTTAATGATCTTGATCT	985-1006	
	FIP*	GTTGGATCCGCTGTTATGTCTTTT	F1c: 856-875	
		GGTCCAAGAGAGAATGTAG	F2: 805-824	
	BIP**	ATGAGAGTGAATTGGAAAAGATGGTTTT	B1: 901-924	
		TGGACATTACCTGTACAATCT	B2c: 962-982	
	Loop F	ATTAGAGCCACCAACTTGTAT	829-849	
	Loop B	GGCAAGTGTTCTATACTAT	926-944	

* FIP (F1c-TTTT-F2) consists of F2 and F1c sequences, **BIP (B1-TTTT-B2c) consists of B1 and B2c sequences as shown on Fig. 1. B2c sequence complementary to B2, F1c sequence complementary to F1.

multiplex nested-PCR. In the first PCR for the identification of G genotype, expected amplicon size of 1062 bp of VP7 (G) gene was obtained in 152 of 154 (98.7 %) positive samples. In the second PCR for differentiating the G genotypes, the expected PCR product of 749 bp size was obtained for the G1. The predominant strain in the population under surveillance was G1, that being identified in 79 of 152 samples (51.97%).

Assay design

The success of LAMP amplification relies on the specificity of designed primer sets. The primers used in this study were selected from a conserved region of VP7 gene of RVA-G1. Our primers can detect all known RVA-G1s available in the GenBank-EMBL database (www.EBI.ac.uk). RVA-G1 LAMP primers, with regard to their positions in the genomic sequences, are shown in Table 1, and a schematic representation of the primer design is depicted in Fig. 1. The LAMP reaction was able to amplify the 228-bp target sequence at constant temperature of 62°C at 90 min.

Detection of LAMP products by alternate methods

According to the principle of LAMP, the band pattern of amplified products in agarose gel electrophoresis verified the LAMP-specific ladder structure, which contains a mixture of stem-loop DNAs with various stem lengths and cauliflower-like structures with multiple loops (Fig. 2(a)). After addition of SYBR Green I to the reaction tube, positive reactions (amplified products) turned green, whereas negative control remained orange. Results obtained with the visual detection methods correlated with the agarose gel elec-

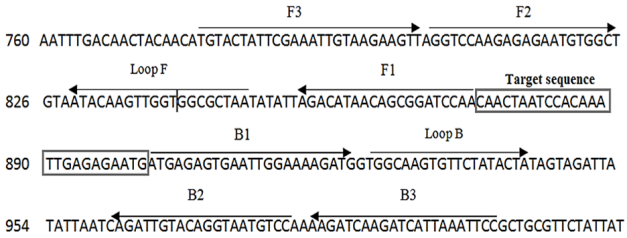
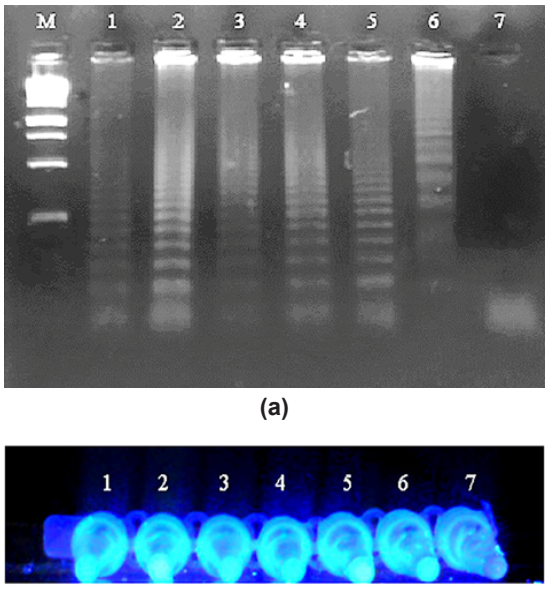


Fig. 1. Diagram of LAMP primers in vp7 region of Rotavirus G1genome RNA (GenBank accession number GU985239.1). The underlined letters indicate the sequences of primers.



(a)

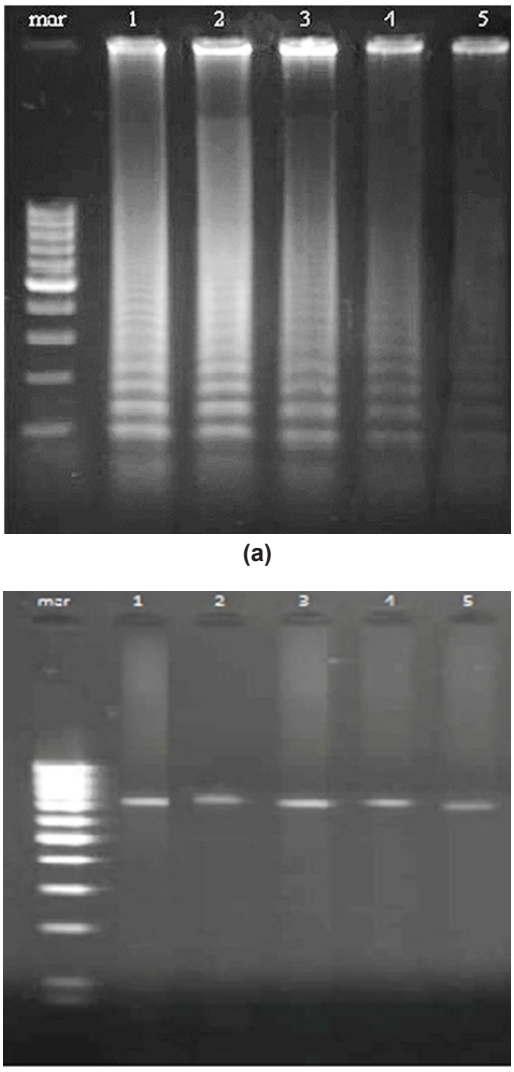
(b)

Fig. 2. (a) Electrophoretic analysis of LAMP amplified products on a 2% agarose gel; lane M 100-bp DNA ladder marker, lane 1–5 LAMP products, lane 6 LAMP Positive control, lane 7 negative controls. (b) SYBR green I fluorescent dye-mediated monitoring of rotavirus LAMP amplification. Tube 1–5 LAMP product, tube 6 Positive control, tube 7 negative control.

trophoresis results (Fig. 2(b)).

Specificity and sensitivity of the LAMP reaction

BLAST analysis of primer sequences confirmed that LAMP primers designed in this study were specifically aimed at recognizing the VP7 sequence of the RVA-G1. In addition, these LAMP primers demonstrated a high degree of specificity for RVA-G1 by amplifying only RVA-G1 template and yielding negative results for other enteric viruses tested Fig. 4. The sensitivity of the RVA-G1 LAMP reaction was tested for 10 samples by using 10-fold serial dilution of a positive control RNA which containing 10^4 copies ml^{-1}, from 10^4 to 10^0 and compared with nested-PCR. Comparative analysis of the sensitivity of RVA-G1 detection by the LAMP and nested-PCR assays showed that both test systems were able to detect as few as 10 copies ml^{-1}. RVA-G1 LAMP reaction was done at 62 ˚C for 90 min and nested-PCR was done 225 min. A 100 % concordance between the two test systems, with regard to sensitivity, was observed. The limits for the RVA-G1 LAMP reaction under optimal conditions (at 62ºC for 90 min) are shown in Figs. (3,4).



(a)

(b)

Fig. 3. (a) LAMP reaction; (b) Nested PCR reaction; lane M 100-bp ladder size markers, lanes 1-5 104, 103, 102, 101 and 100 copies ml^{-1}

DISCUSION

Over past decades, RVA-G1 has been the most prevalent genotype responsible for the hospitalization of children from acute diarrhea worldwide [23-25]. The most prevalent cause of severe gastroenteritis in infants is *Rotavirus* (RV) [26]. *Rotavirus* diarrheal illnesses can be mild or produce severe dehydration leading to hospitalization and mortality if not treated [27,28]. This virus is the eighth leading cause of death among all ages and the fifth leading cause of death for children under 5 in the world. About 3 to 5 million children are infected each year, and about 446,000 of them die [29]. Also, nosocomial *rotavirus* (nRV)

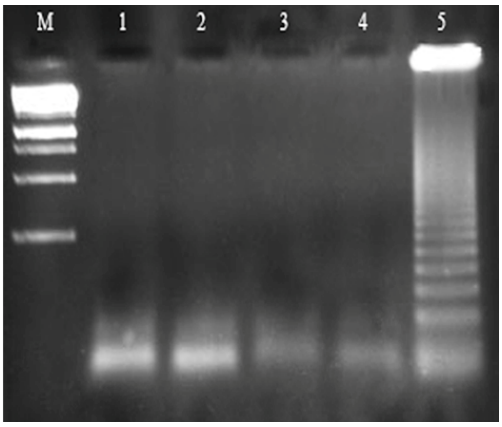


Fig. 4. Agarose gel illustrating the specificity of the designed primers to G1 rotavirus RNA; lane M 100 bp ladder, lanes 1-4 genome of adenovirus group F, norwalk virus, sapovirus and Negative control, lane 5 LAMP product of rotavirus RNA.

infections are an important problem among hospitalized infants during the winter months [30]. Rapid and accurate diagnosis of *Rotavirus* may be valuable in the hospital environment for the rapid identification of infected individuals so that they can be properly isolated to prevent transmission of infection to others and ensure adequate treatment. Currently, both molecular and immunological methods are highly sensitive and rapid assays for routine laboratory diagnosis of *Rotavirus* in stool samples [31]. There are many techniques for detecting *rotavirus*. At first, *rotavirus* was diagnosed using an electron microscope. After that, polyacrylamide gel electrophoresis (PAGE), immune-fluorescence (IF), radioimmune- assay (RIA), reverse passive hemagglutination (RPH), enzyme- linked immunosorbent (ELISA) Latex agglutination test (LA) and, recently reverse transcription-polymerase chain reaction (RT- PCR) and immunochromatography (IMC) techniques have also been used [32]. Compared to molecular and immunological assays, the LAMP approach is significantly simpler to perform and its detection ability is more sensitive [33]. Extensive use of the lamp method for accurate molecular diagnosis in many human-related diseases shows its high potential [34]. An important advantage of LAMP is that the thermal-cycling needs of the PCR method are completely avoided and the reaction can be conducted with a simple heating block [17,35]. In addition, the specificity of the reaction is extremely high because it uses six primers that recognize six or eight distinct regions on the target sequence [34,36]. Therefore, the present study was aimed to development of LAMP method for routine detection of RVA-G1 from stool sample, our designed primers, optimized amplification conditions (reaction time, reaction temperature) and testing its specificity and sensitivity. In a similar study by Lin Yu et al, The results showed that the reaction takes place over a period of 30 minutes at 65°C, and this time has been reduced by optimizing the concentration of the reactants and the dose of the virus [37]. The optimal amplification condition for RVA-G1 was at 62°C for 90 min, and the standard heat block was the only equipment. Among the 365 stool samples, 154 cases were found to be positive for RVA by ELISA method, and 152 (42.2%) of them were G typed by nested-multiplex PCR. We found genotype G1 predominant and identified in 79 (51.97%) of 152 samples. All G1 typed cases were found to be positive by LAMP assay; two positive cases that detected by ELISA, were non-typeable in nested-PCR and negative by LAMP procedure, too. Our results demonstrated that LAMP has a high sensitivity, equal to those for the nested-PCR method and detection limit of both systems was 10 copies ml^{-1}. The LAMP primers used in this research have been described for the first time. As expected, our primers and the developed method were able to detect a large part of the RVA-G1s that was registered in NCBI. Our primers also demonstrated a high degree of specificity for RVA-G1, yielding no cross-reactivity with any other enteric viruses tested. Another study by Keerti Kaumudee Dixit et al, Confirmed the sensitivity and specificity of LAMP using SYBR Green I [38]. Use of SYBR Green I for visual inspection of LAMP amplification products was a simple and superior technique and post-amplification processes such as electrophoresis and ethidium bromide staining were obviated. Although the sensitivity of detecting RVA-G1 by the LAMP reaction was equivalent to the nested-PCR test, it is considered superior because it is a simpler, cost- and time-effective procedure. The ability to obtain accurate results within 90 min after extraction of the viral genome and cDNA synthesis is particularly amenable to private clinics and hospital-based settings. In conclusion, the present study confirmed that LAMP is simple, rapid, and cost effective as well as highly sensitive and specific assay.

This has potential usefulness for clinical diagnosis of RVA-G1, as it does not require the use of sophisticated equipment or skilled personnel.

ACKNOWLEDGEMENTS

We are thankful to the Islamic Azad University, Jahrom and Shahrekord branches. We also acknowledge the cooperation of Bushehr and Shiraz University of Medical Sciences.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES

[1] Mokomane, M., Kasvosve, I., Melo, Ed., Pernica, J.M., Goldfarb, D.M. (2017). The global problem of childhood diarrhoeal diseases: emerging strategies in prevention and management. TAI, 5(1): 29-43.

[2] Zaraket, R., Salami, A., Bahmad, M., El Roz, A., Khalaf, B., Ghssein, G. (2020). Prevalence, risk factors, and clinical characteristics of *rotavirus* and adenovirus among Lebanese hospitalized children with acute gastroenteritis. Heliyon, 6(6): e04248.

[3] Crawford, S.E., Ramani, S., Tate, J.E., Parashar, U.D., Svensson, L., Hagbom, M. (2017). Rotavirus infection. Nat. Rev. Dis. Primers, 3(1): 17083.

[4] Souvik Ghosh, N.K. (2014). Genetic Diversity and Evolution of Human Rotaviruses Based on Whole Genome.

[5] Ouermi, D., Soubeiga, D., Nadembega, W.M.C., Sawadogo, P.M., Zohoncon, T.M., Obiri-Yeboah, D. (2017). Molecular Epidemiology of Rotavirus in Children under Five in Africa (2006-2016): A Systematic Review. Pak J Biol Sci., 20(2): 59-69.

[6] Tavakoli Nick, S., Mohebbi, S.R., Ghaemi, A., Hosseini, S.M. (2019). Human rotavirus in Iran; molecular epidemiology, genetic diversity and recent updates on vaccine advances. Gastroenterol. Hepatol. Bed Bench, 12(2): 98-109.

[7] Santos, N., Hoshino, Y. (2005). Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. R Rev. Med. Virol, 15(1): 29-56.

[8] Azaran, A., Makvandi, M., Teimoori, A., Ebrahimi, S., Heydari, F., Nikfar, R. (2018). Distribution of Rotavirus Genotypes Ccirculating in Ahvaz, Iran in 2016. Iran Biomed J., 22(2): 107-16.

[9] Tafalla, M., Gardovska, D., Gopala, K., Kozlovska, L. (2019). Primary care-based surveillance to estimate the proportion of rotavirus gastroenteritis among Latvian children below 5 years of age with acute gastroenteritis. Hum. Vaccines Immunother, 15(6): 1272-8.

[10] Nisa, T.U. (2019). Rotavirus genotyping: a promising diagnostic tool of research for pediatric gastroenteritis. Pak. j. med, 8(3): 83-86.

[11] Logan, C., O'Leary, J.J., O'Sullivan, N. (2006). Real-time reverse transcription-PCR for detection of rotavirus and adenovirus as causative agents of acute viral gastroenteritis in children. J. Clin. Microbiol, 44(9): 3189-95.

[12] Organization, W.H. (2009). Manual of rotavirus detection and characterization methods. World Health Organization.

[13] De La Cruz Hernández, S.I., Anaya Molina, Y., Gómez Santiago, F., Terán Vega, H.L., Monroy Leyva, E., Méndez Pérez, H. (2018). Real-time RT-PCR, a necessary tool to support the diagnosis and surveillance of rotavirus in Mexico. Diagn. Microbiol. Infect. Dis, 90(4): 272-6.

[14] Mousavi-Nasab, S.D., Sabahi, F., Kaghazian, H., Paryan, M., Mirab Samiee, S., Ghaderi, M. (2020). A Real-Time RT-PCR Assay for Genotyping of Rotavirus. Iran Biomed J., 24(6): 399-404.

[15] Barua, S.R., Islam, S., Siddiki, A.Z., Masuduzzaman, M., Hossain, M.A., Chowdhury, S. (2021). Comparison of diagnostic tests for detection of bovine Rotavirus A in calf feces, Mac Vet Rev., 44 (1): 37-45.

[16] Khan, R., Ali, W., Kiran, S., Shah, M., Tahir, Z., Habib, M. (2018). Rapid detection of infectious bursal disease by loop-mediated isothermal am-

plification for field analysis. I Iran. J. Vet. Sci, 19(2): 101.

[17] Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N. (2000). Loop-mediated isothermal amplification of DNA. Nucleic Acids Res, 28(12): e63-e.

[18] Wong, Y.P., Othman, S., Lau, Y.L., Radu, S., Chee, H.Y. (2018). Loop-mediated isothermal amplification (LAMP): a versatile technique for detection of micro-organisms. J. Appl. Microbiol, 124(3): 626-43.

[19] Li, Y., Fan, P., Zhou, S., Zhang, L. (2017). Loop-mediated isothermal amplification (LAMP): a novel rapid detection platform for pathogens. Microb. Pathog, 107: 54-61.

[20] Wang, Y., Wang, C., Wang, L., Zhang, X., Yan, J., Wang, J. (2020). Development of loop-mediated isothermal amplification (LAMP) assay for rapid detection of Fusarium proliferatum causing ear and kernel rot on maize. Crop Protection, 132(6): 105142.

[21] Silva, SJRd., Pardee K., Pena, L. (2020). Loop-mediated isothermal amplification (LAMP) for the diagnosis of Zika virus: A Review. Viruses, 12(1): 19.

[22] Gouvea, V., Glass, R.I., Woods, P., Taniguchi, K., Clark, H.F., Forrester, B. (1990). Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J. Clin. Microbiol, 28(2): 276-82.

[23] Zhou, N., Zhou, L., Wang, B. (2020). Genetic Characterizations and Molecular Evolution of VP7 Gene in Human Group A Rotavirus G1. Viruses, 12(8): 831.

[24] Kowalzik, F., Zepp, F., Hoffmann, I., Binder, H., Lautz, D., Van Ewijk, R. (2016). Disease Burden of Rotavirus Gastroenteritis in Children Residing in Germany: A Retrospective, Hospital-based Surveillance. Pediatr. Infect. Dis. J, 35(1): 97-103.

[25] Lorrot, M., Bon, F., El Hajje, M.J., Aho, S., Wolfer, M., Giraudon, H. (2011). Epidemiology and clinical features of gastroenteritis in hospitalised children: prospective survey during a 2-year period in a Parisian hospital, France. Eur. J. Clin. Microbiol. Infect. Dis, 30(3): 361-8.

[26] Chiu, M., Bao, C., Sadarangani, M. (2019). Dilemmas With Rotavirus Vaccine: The Neonate and Immunocompromised. Pediatr. Infect. Dis. J, 38(6S): S43-S6.

[27] Staat, M.A., Azimi, P.H., Berke, T., Roberts, N., Bernstein, D.I., Ward, R.L., Pickering, L.K., Matson, D.O. (2002). Clinical presentations of rotavirus infection among hospitalized children. Pediatr Infect Dis J., 21(3): 221–7.

[28] Clark, B., McKendrick, M. (2004). A review of viral gastroenteritis. Curr Opin Infect Dis, 17(5): 461-9.

[29] Kozishkurt, EV. (2019). Prevalence of acute intestinal infections and their role in human pathology (review). J. Phys. Educ, 9(9): 1308-1323.

[30] Bruijning-Verhagen, P., Quach, C., Bonten, M. (2012). Nosocomial Rotavirus Infections: A Meta-analysis. Pediatrics, 129(4): e1011-9.

[31] Desselberger, U. (2000). Rotaviruses: Basic Facts. In: Gray J, Desselberger U, editors. Rotaviruses: Methods and Protocols. Totowa, NJ: Humana Press, p. 1-8.

[32] Hamzavi, H., Azaran, A., Makvandi, M., Karami, S., Roayaei Ardakani, M., Mozaffari Nejad, A.S. (2018). Performance of Latex agglutination, ELISA and RT-PCR for diagnosis of Rotavirus infection. J. Biol. Res, 90(2).

[33] Becherer, L. (2020). Loop-mediated isothermal amplification (LAMP) – review and classification of methods for sequence-specific detection. Anal. Methods, 12(6): 717-46.

[34] Wong, Y.P., Othman, S., Lau, Y.L., Radu, S., Chee, H.Y. (2018). Loop-mediated isothermal amplification (LAMP): a versatile technique for detection of micro-organisms. J. Appl. Microbiol, 124(3): 626-43.

[35] Parida, M., Sannarangaiah, S., Dash, P.K., Rao, PVL., Morita, K. (2008). Loop mediated isothermal amplification (LAMP): a new generation of innovative gene amplification technique; perspectives in clinical diagnosis of infectious diseases. Rev. Med. Virol, 18(6): 407-21.

[36] Nagamine, K., Hase, T., Notomi, T. (2002). Accelerated reaction by loop-mediated isothermal amplification using loop primers. Mol Cell Probes, 16(3): 223-9.

[37] Yu, L., Wu, S., Hao, X., Dong, X., Mao, L., Pelechano, V. (2020). Rapid Detection of COVID-19 Coronavirus Using a Reverse Transcriptional Loop-Mediated Isothermal Amplification (RT-LAMP) Diagnostic Platform. Clin Chem., 66(7): 975-7.

[38] Dixit, K.K., Verma, S., Singh, O.P., Singh, D., Singh, A.P., Gupta, R. (2018). Validation of SYBR green I based closed tube loop mediated isothermal amplification (LAMP) assay and simplified direct-blood-lysis (DBL)-LAMP assay for diagnosis of visceral leishmaniasis (VL). PLoS Negl Trop Dis., 12(11): e0006922-e.

AUTHOR (S) BIOSKETCHES

Mohammad Kargar, Professor of Microbiology, Department of Microbiology, Jahrom Branch, Islamic Azad University, Jahrom, Iran

Neda Hoveizeh Tamimian, M.Sc., Department of Biology, Islamic Azad University Tehran North Branch, Tehran, Iran, *Email: neda.tamimian@gmail.com*

Abbas Doosti, Associate Professor, Biotechnology Research Center, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

Farnaz Moghadamdizajherik, M.Sc., Department of Immunology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Fatemeh Mazaheri-Eftekhar, M.Sc., Department of Nutrition, Science and Research Branch, Islamic Azad University, Tehran, Iran