JBCS 30 years

http://dx.doi.org/10.21577/0103-5053.20190092

Article

J. Braz. Chem. Soc., Vol. 30, No. 9, 1841-1849, 2019
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Molecular Diagnostics of Dengue by Reverse Transcription-Loop Mediated Isothermal Amplification (RT-LAMP) in Disposable Polyester-Toner Microdevices

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Dengue is one of the most prevalent infectious tropical diseases in the world, with high incidence in over 100 countries. Rapid and reliable diagnosis of dengue is of great importance for public health. To simplify molecular diagnostics, isothermal amplification techniques have recently emerged as an alternative to conventional methods of deoxyribonucleic acid (DNA) amplification. Here, we developed a one-step method for dengue virus detection from real sample based on RT-LAMP (reverse transcription-loop mediated isothermal amplification) in a disposable microdevice. The reaction was thermally controlled with a thermoblock for 15 min at 72 °C. At the end of the incubation time, we either removed the solution for detection of fragments by gel electrophoresis or added DNA intercalator for visual detection on-chip. Our results demonstrated that it is possible to detect dengue virus through RT-LAMP directly from a serum sample, without previous ribonucleic acid (RNA) extraction. The success of RT-LAMP was confirmed in reactions initiated with 0.8 fg μL^{-1} of RNA, which represents 200 copies of RNA *per* µL. RT-LAMP in a polyester-toner (PeT) microdevice is a simple and inexpensive method that allows for rapid detection of dengue virus with high reliability and great potential for point-of-care applications.

Keywords: RT-LAMP, PeT microdevice, dengue virus, molecular diagnosis, point-of-care

Introduction

Dengue virus (DENV) is the most widespread arbovirus in the world with occurrence in more than 100 countries in tropical and subtropical regions of the world. The World Health Organization (WHO) estimates that about 390 million infections (96 million symptomatic) and 20,000 deaths occur every year.[1,2] The reemergence of sudden epidemics has been a severe problem in global health since the early 20th century.[3,4]

Rapid and efficient detection of the infection is crucial for suitable treatment as well as for appropriate control of the disease.[5] Detection of virus-specific antibodies and virus isolation are the most commonly applied diagnosis tests in resource-limited laboratories. Detection based on the NS1 antigen is concluded within five days of infection, though it is unable to differentiate serotypes.[5,6] ELISA (enzyme-linked immunosorbent assay), an immunoenzymatic test based on the detection of IgG and IgM antibodies, is the most widespread serological method for diagnosis of dengue. However, depending on the stage of infection, there may not yet be a significant increase in these antibodies. A more significant amount of sample is required to confirm the results, and the cross-reactivity of antibodies to other

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flaviviruses can be a possible source of false-positives.[7] Virus isolation, which is the most consolidated procedure and considered as the gold standard, is a high-cost test that needs seven days to complete.[8-10] Besides that, these methods are not able to distinguish the serotypes of the viruses.

Molecular diagnostics based on nucleic acid amplification are very sensitive and specific in addition to being able to distinguish the different serotypes, which is essential for epidemiological surveillance. Polymerase chain reaction (PCR), the most used amplification method, presents disadvantages such as the need for thermal cycling heating and time-consuming detection. Thus, PCR tests require sophisticated equipment and the adaptation to routine clinical use is not easy, particularly in limited resource laboratories.[1,5,6,11,12]

To eliminate complex and costly protocols, the use of isothermal amplification techniques has emerged as a promising molecular diagnostic tool. Currently, several isothermal methods towards dengue molecular diagnostics include nucleic acid sequence based amplification (NASBA),[13,14] reverse transcription recombinase polymerase amplification (RT-RPA),[15,16] and reverse transcription loop mediated isothermal amplification (RT-LAMP).[17,18] Among them, loop mediated isothermal amplification (LAMP) is a technique with a great potential to overcome PCR limitations.[19-23]

LAMP relies on the displacement of strands by the action of a Bst DNA polymerase (*Bacillus stearothermophilus* DNA polymerase) enzyme and primers designed to recognize six distinct regions of a target gene/unique sequence. Other advantages, such as sensitivity, specificity, cost-effective instrumentation, and less time consumption, contribute to the use of LAMP in molecular diagnostics.[24-27] Furthermore, LAMP products can be detected quickly by visual detection through turbidity,[27,28] lateral flow,[29,30] metal precipitation,[27,31] and fluorescence using a deoxyribonucleic acid (DNA) intercalator dye.[12,32]

The RT-LAMP assay has been successfully used for molecular dengue diagnostics.[12,33] Parida *et al.*[12] first described this method, using a combination of the two enzymes, Bst DNA polymerase and reverse transcriptase, to develop a single-step test for ribonucleic acid (RNA) amplification of dengue virus in a tube containing 25 µL of solution with 60 min of heating.

In recent years, microfluidics has demonstrated many applications for clinical diagnostics with a high potential toward point-of-care applications.[34-36] High analytical performance, ease of system integration, improved automation potential, use of small volumes of samples and reagents, cost-effective setup, high sensitivity detection, and reduced analysis times compared to bench-top size analyses characterize these microfluidic platforms.[37-39]

Polyester-toner (PeT) microchips have been widely used in recent years for applications with biological samples, thus demonstrating compatibility for clinical diagnostic applications.[40-50] Both polyester and toner are inexpensive materials and the microfabrication process of the device is simple, fast, and low-cost, in addition to being disposable.[41]

In this paper, we describe a simple and rapid method for molecular diagnostics of dengue fever by RT-LAMP in a PeT microchip. For a proof-of-concept, we detected the DENV-4 in serum samples of infected patients. The reaction was developed using a simple heating block and on-chip visual detection using SYBR Green I intercalator, aided by a hand-held UV source and images obtained by smartphone.

Experimental

Human patient serum samples

Serum samples were obtained from patients with confirmed dengue virus (DENV-4) infections during epidemics in Brazil in 2013. A confirmed case of dengue virus infection was defined as a febrile illness associated with isolation of dengue virus and positive reverse transcription-polymerase chain reaction (RT-PCR) results. This study was approved by the Research Ethics Committee of the Hospital Materno Infantil, with protocol No. 17/2012, as well as by the Teaching and Research Sector of the Municipal Health Department of Goiânia, which authorized the study in the public health units. All experiments were performed in compliance with either nationally required guidelines, following the resolutions (National Health Council): CNS 466/12 and CNS 441/11, and in compliance with institutional guidelines. Furthermore, consent was obtained from all patients.

Nucleic acid extraction, cDNA synthesis and qPCR

For the quantification of viral load, RNA from human serum samples was extracted by dynamic solid-phase RNA extraction according to the protocol previously described by Gimenez *et al.*[40] The RNA was eluted in RNase free water and stored at −80 °C. The viral RNA was reverse transcribed using a specific primer R (5'-TCCACCTGAGACTCCTTCCA-3') with SuperScript® III Reverse Transcriptase (Invitrogen, Van Allen Way, Carlsbad, USA). The reverse transcription reaction was carried out at 48 °C for 1 h. The cDNA thus obtained was

used as the template for the quantitative PCR (qPCR). The PoweUp™ SYBR® Green Master Mix (Applied Biosystems, Austin city, USA) was used in all qPCRs. Each reaction had 40 nmol L^{-1} of forward primer (R: 5'-TCCACCTGAGACTCCTTCCA-3') and 40 nmol L^{-1} of reverse primer (F: 5'-TTGTCCTAATGATGCTAGTCG-3') in a 20 µL of final volume. The PCR mixtures were incubated at 95 °C for 10 min, 40 cycles of 95 °C for 15 s and 60 °C for 1 min, using the Applied Biosystems Life technologies real-time PCR system™ (ThermoFisher, Foster City, USA). Serial dilution of gBlock® (5'-TTGTCCTAATGATGCTAGTCGCCCCATCC TACGGAATGCGATGCGTAGGGGTGGGGAACA GAGACTTTGTGGAAGGAGTCTCAGGTGGA-3') gene fragments (Integrated DNA Technologies, IA, USA) was used to generate a standard curve (SC) for absolute quantification (20 to 2×10^4 copies of viral RNA) and cycle threshold (Ct) values. The real-time data was analyzed using the StepOnePlus™ System provided by Applied Biosystems (Carlsbad, USA).

Fabrication of the PeT microdevice

PeT microchips were fabricated using a print, cut, and laminate (PCL) protocol previously described.[42] The bottom and top layers of the microdevice are polyester films with precut access holes in the top sheet only. The intermediated layers are polyester sheets covered with toner on both sides using a laser printer (Hewlett-Packard 1102w). The design of the chamber was drawn using CorelDraw 11.0 software,[51] and the chamber was created by cutting the microchannel out with a 50-watt CO_2 laser cutter (Red Sail Laser/M550). The microchamber was designed to be 7-mm long, 2-mm wide, and 350-µm deep yielding a total inner volume of 5 µL. The bottom, top, and three intermediated layers were aligned and laminated together using an office laminator (230c A4) at 160 °C.

RT-LAMP amplification of dengue virus in PeT microchip

The sequences of primers used for RT-LAMP on DENV-4 detection, which has been described by Hu *et al.*,[52] are shown in Table 1.

The RT-LAMP master mixture with 5 µL of total volume contained: 0.1 µmol L^{-1} of each outer primer (F3 e B3), 1.6 µmol L^{-1} of each inner primer (FIP e BIP), 0.8 µmol L^{-1} of each loop primer (LFP e LBP), 6 mmol L^{-1} $MgSO_4$, 1.4 mmol L^{-1} dNTP (deoxyribonucleotide triphosphate) , 0.24 mg mL^{-1} BSA (bovine serum albumin), 0.96 U μL^{-1} of Bst 2.0 or 0.64 U μL^{-1} of Bst 3.0 polymerase, 0.5 µL of 10 × isothermal amplification buffer (20 mmol L^{-1} tris-HCl, 10 mmol L^{-1} KCl, 10 mmol L^{-1} $(NH_4)_2SO_4$, 2 mmol L^{-1} $MgSO_4$, 0.1% triton X-100 (Usb Corporation, Cleveland, USA) and varying amounts of RNA or serum sample. In reactions using Bst 2.0, the addition of 0.024 mmol L^{-1} DTT (dithiothreitol, Promega, Madison, USA), 1.6 U μL^{-1} SuperScript® III Reverse Transcriptase and RNase OUT™ (Promega, Madison, USA) was necessary. Before use, the microchambers were passivated with BSA (1.0 mg mL^{-1}), as previously described.[41] After passivation, the LAMP chamber was filled with approximately 5 µL of master mix solution by capillary force. Mineral oil overlaid on both reservoirs prevented evaporation of the solution. The positive reaction and negative control (lacking dengue virus RNA) were placed in a thermoblock (Major Science, Saratoga, CA) at 72 °C for 15 min, followed by heating at 80 °C for 2 min to deactivate the enzyme. At the end of the reaction incubation time, either the solution was removed from the microchip via manual pipetting for gel electrophoresis or 0.5 µL of the fluorescent DNA intercalator SYBR Green I (Sigma-Aldrich, Saint Louis, USA) was added for visual detection on-chip, as shown in the scheme of Figure 1.

Table 1. Sequences of primers for RT-LAMP

Primer	5' to 3'
F3	GCTCCTTTCGAGAGTGAAG
B3	AGTACAGCTTCCTCCTGG
FIP	CGTTATTGGCGGAGCTACAGGGAGGCTATTGAAGTCAGGC
BIP	GGAGGCGTTAAATTCCCAGGGGTCTCCTCTAACCGCTAGT
LFP	CAGCACGGTTTGCTCAAG
LBP	CTGTACGCGTGGCATATTG

Analysis of RT-LAMP products by gel electrophoresis

For off-chip detection, gel electrophoresis using 2% agarose gel in 0.5% tris-borate-EDTA (TBE) buffer separated the amplified DNA. The electrophoretic run was carried out in 0.5% TBE buffer with 90 V potential conditions, at times ranging from 30 to 115 min. After the running time, the DNA bands were visualized through a UV transilluminator coupled to a photodocumentation system (GE Healthcare LifeSciences, Marlborough, USA).

Visual detection

For on-chip detection, 0.5 µL of SYBR Green I (1:10) was directly added to the microchamber at the end of the reaction. The reaction chamber was exposed to a UV lamp illumination (320 nm), and images were taken with a smartphone.

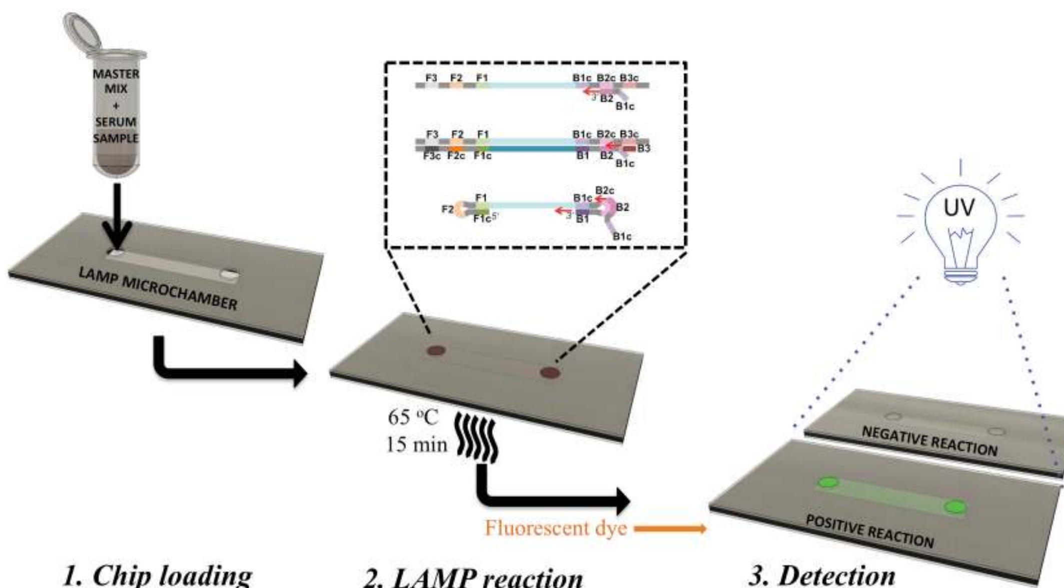

Figure 1. Schematic illustration of the RT-LAMP amplification and detection main steps in PeT microdevice.

Results and Discussion

We previously demonstrated that PeT devices could be successfully used for DNA amplification by LAMP.[41] In our first paper about isothermal DNA amplification in a PeT microchip, we demonstrated *E. coli* detection with high sensitivity. We also confirmed that it is necessary to passivate the inner surfaces of the channels to avoid the absorption of reagents on microchamber. The passivation of the polyester surface of the microchamber for DNA amplification is now well established and is also demonstrated by other studies.[45] LAMP amplification on PeT microchip confirmed that polyester surface passivation improves the efficiency of the amplification reaction. The best results obtained previously required passivation with BSA (0.6 mg mL^{-1}). In our previous study,[41] we also found the importance of controlling the A / V (area / volume) to improve the efficiency of the amplification reaction. The results showed that the best A / V ratio was 6 mm^2 μL^{-1} (channel dimensions: 7 mm in length, 2 mm in width and 0.35 mm in depth). The chamber in these dimensions has a capacity of 5 µL for a reaction solution. Here, we adapted our previous method[41] described for DNA detection with LAMP and developed for the first time the RT-LAMP for detection of RNA virus in a PeT microchip using real samples from patients infected with DENV-4. Alternative molecular diagnosis for pathogen detection involving RNA in biological samples has vital importance for public health, because there are numerous pathogens whose genomes consist of RNA, as with many viruses (e.g., dengue virus, zika virus, influenza, and human immunodeficiency virus (HIV)). RNA analysis is more complicated than DNA analysis because RNA is less stable than DNA. Despite challenges of RNA analysis, our results showed that RNA is compatible with substrates used in microchip fabrication (i.e., polyester and toner), and RNA was not degraded by RNases in our protocol.

Besides that, molecular diagnosis involving DNA amplification from an RNA sample requires the reverse transcription step to convert the RNA to DNA. In this work, we performed the conversion of RNA to DNA and DNA amplification in a single step, followed by visual detection in a PeT microdevice.

Bst 2.0 × Bst 3.0 DNA polymerases

LAMP uses a strand-displacement DNA polymerase enzyme, Bst DNA polymerase, that is the portion of the *Bacillus stearothermophilus* DNA polymerase protein which contains the 5' → 3' polymerase activity, but lacks 5' → 3' exonuclease activity. Currently, some other versions of Bst DNA polymerase are available. We started our experiments using the 2.0 version. The Bst 2.0 DNA polymerase displays improved amplification speed, yield, salt tolerance, and thermostability compared to wild-type Bst DNA polymerase. The Bst 2.0 developed for LAMP reactions can be used for RT-LAMP reactions as long as a reverse transcriptase enzyme is added. Detection of RNA targets is accomplished by simple addition of reverse transcriptase to the LAMP reaction, with RT-LAMP performed as a true one-step isothermal workflow. Another Bst version developed for RT-LAMP, Bst 3.0, features further improvements in amplification speed, inhibitor tolerance, thermostability, and dNTP incorporation. Bst 3.0

also displays significantly higher reverse transcriptase activity than the previous version and can be used for single-enzyme RT-LAMP reactions.

As dengue virus is an RNA virus, using the Bst 2.0 requires an extra transcriptase enzyme in the master mix. When we switched to Bst 3.0, it was no longer necessary. The experiments using both enzymes showed that the initial concentration of Bst 2.0 (0.96 U μL^{-1}) required to produce a detectable amount of DNA on an agarose gel or visual detection could be decreased by 0.64 U μL^{-1}, indicating that a lower concentration of this enzyme is enough due to its strong strand displacement activity. Furthermore, we observed that after 15 min of reaction it is already possible to observe detectable amounts of DNA on the agarose gel when the enzyme Bst 3.0 was used. In contrast, using the Bst 2.0 required 60 min of incubation time to allow for the visualization of detectable amounts of DNA on agarose gel (Figure 2). Therefore, the use of the enzyme Bst 3.0 lowers the cost of the reaction, since it does not require the use of an extra transcriptase enzyme, it needs a lower enzyme concentration, and it also provides results in shorter analysis times. As Bst 3.0 provides the fastest amplification time, it is an ideal candidate for rapid diagnostic methods.

Limit of detection of RT-LAMP for RNA dengue virus from serum sample in PeT microdevices

In order to evaluate the limit of detection of dengue virus by RT-LAMP in PeT microchip, the amplification was carried out with serum samples of patients infected with DENV-4, which was previously quantified by qPCR. The average of viral load of the samples was 7200 RNA copies μL^{-1} on the third day of fever. The reactions with serum samples started from initial copies of RNA ranging from 2×10^4 down to 500 *per* reaction by serial dilution of the serum. The results showed that the PeT platform allowed detection of amplicons on the agarose gel in reactions starting with 200 copies of RNA μL^{-1} or 1000 copies of RNA *per* reaction, which means 0.8 fg μL^{-1} of RNA in the master mixture (Figure 3).

In our previous study on the LAMP amplification in PeT microchips we found a limit of detection of 1 fg μL^{-1} of *E. coli* DNA in 60 min reaction.[41] In the present study, we performed RT-LAMP amplification directly from serum samples without RNA pre-purification in 15 min reaction with a limit of detection of 0.8 fg μL^{-1} or 24.3 fM.

Other RT-LAMP studies for the detection of dengue virus found lower limits of detection, but most of them were performed from the pre-purified RNA sample and generally with longer reaction times. Our limit of detection of target RNA in the serum sample may be related to the presence of proteins, ribonucleases (RNases) and other compounds present in serum samples from human patients, compared to synthetic RNA / DNA.[53,54]

Kim *et al.*[55] detected DENV virus from purified RNA samples in 25 min of incubation in microtubes with limit of detection of 3.5 copies μL^{-1}. Lo *et al.*[56] detected the amplicons from RT-LAMP on paper-based devices, having

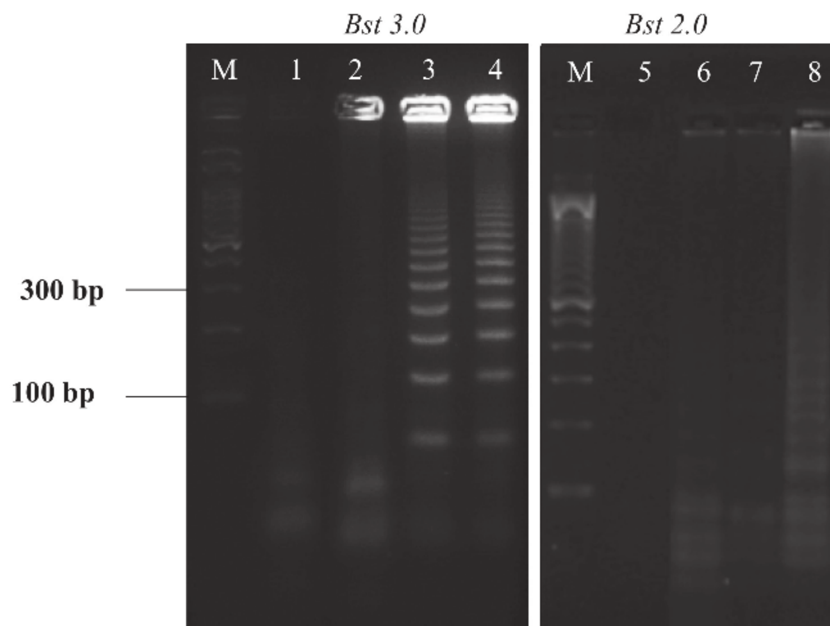

Figure 2. Monitoring by gel electrophoresis of the success amplification using serum from patients infected with DENV-4 by RT-LAMP in PeT microdevices with the Bst 2.0 and Bst 3.0 enzymes at different reaction heating times. In both panels: (M) 1 kb Invitrogen DNA ladder; (1) negative control with heating of 15 min; 2-4 serum samples with heating of: (2) 10 min, (3) 15 min, (4) 20 min; (5) negative control with heating of 60 min; 6-8 serum samples with heating of: (6) 30 min, (7) 45 min, (8) 60 min.

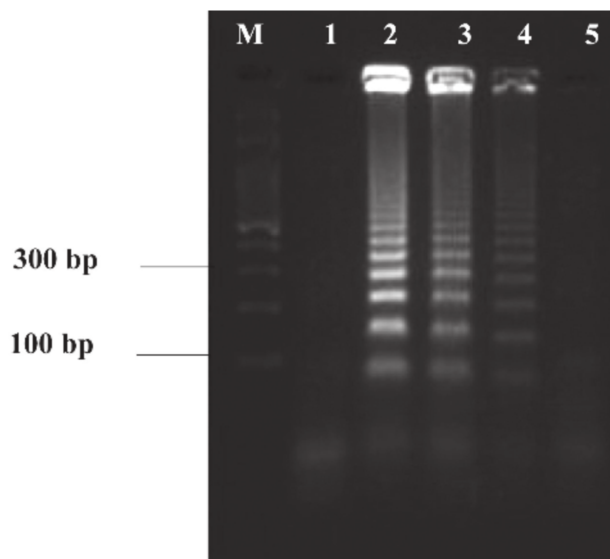

Figure 3. Agarose gel eletrophoresis determining the limit of detection performed off-chip for DENV-4 analysis by RT-LAMP. Lanes: (M) 1 kb Invitrogen DNA ladder; (1) negative control (serum sample of healthy patient); (2) 10000 copies; (3) 5000 copies; (4) 1000 copies; (5) 500 copies of RNA from serum sample of infected patients.

RNA dengue virus as the target, and the limit of detection obtained was 300 ng mL^{-1} in reactions lasting 60 min. In 2015, Lau *et al.*[27] reported a single-tube RT-LAMP assay detecting 10 RNA copies μL^{-1} of DENV in 45-min reactions. Lu *et al.*[33] detected 12 copies of RNA dengue virus *per* reaction within 90 min of RT-LAMP reaction.

Although the limit of detection obtained here is not as low as those from other methods, our method presents clear advantages. We can highlight (*i*) low volume of sample (0.2 µL of serum sample *per* reaction), (*ii*) low reagent consumption (< 5 µL), (*iii*) short reaction time (15 min), (*iv*) no RNA extraction, (*v*) visual detection directly on-chip, (*vi*) low cost (less than US$ 1) disposable device, (*vii*) suitable for applications at poin-of-care. Such characteristics make our methodology highly attractive for molecular diagnostics of dengue, since this limit of detection (1000 copies) is sufficient to carry out a reliable diagnosis of the disease.

Molecular methods in portable platforms are an important tool in early diagnosis of dengue because they are fast, accurate, and low-cost. Our molecular diagnosis using the RT-LAMP technique is able to detect the virus even in samples with relatively low viral load. Therefore, it presents relevant applicability in the differential diagnosis of infection by other arboviruses such as zika and chikungunya, which is currently considered the greatest clinical difficulty due to the similarity of the clinical symptoms. The early diagnosis of dengue can aid in the adequate treatment of the disease and in reducing the mortality rate of patients before the onset of complications. Early diagnosis means detecting the disease at the initial stage of progression. In general, the mean duration of viremia is seven days, however, the maximum viral load is observed between the first and third days of the onset of symptoms.[57] Although the interference of different factors in the progression of the infection, related to the virus or host, during the first three days of infection the viral load remains relatively constant, having an average value of 1.0×10^9 RNA copies mL^{-1}, regardless of the serotype.[58] Considering this mean value of RNA copies found in the infected host serum in the first day of symptoms, only 0.001 µL of patient serum *per* reaction / chip would be needed. Thus, even if the host had a viral load much lower than the mean value usually found at the beginning of the infection (as was the case of the sample evaluated in this study, 7.2×10^6 copies mL^{-1}), 0.2 µL of serum is more than enough. Therefore, RT-LAMP is an important tool that can be performed on samples collected immediately after the onset of symptoms, allowing the diagnosis in the early stages when the serological methods are still negative.

An important disadvantage of LAMP is that it is subject to contamination of the amplicon, generating false-positive results. In order to ensure correct results, appropriate contamination control procedures should be used. In this work different equipment and laboratory environments were used for negative and positive reactions. In this way, we eliminated, or reduced to minimum, false-positive results.

Visual detection

As an alternative to off-chip detection by gel electrophoresis, which takes a long time (about 60 min) to obtain the final result, we perform on-chip visual detection using a DNA intercalator. In the presence of DNA, the solution exhibits bright green fluorescence after the addition of the intercalator (SYBER Green I). The on-chip detection reduced the detection time from 60 down to 2 min without the need for sophisticated equipment, using only a UV-light source and a smartphone for image capture. The fluorescence intensity of solution decreases with decreasing RNA initial concentration, as shown in Figure 4. The visual detection shown in Figure 4 was performed from serum samples from patients infected with DENV-4 with serial dilution ranging from 2×10^4 to 500 copies of RNA *per* reaction. The visual detection on-chip allowed the confirmation of positive reactions with up to 1000 copies of RNA *per* reaction (0.8 fg μL^{-1} of RNA on master mixture). These results coincide with the intensity of the amplified DNA fragments shown by electrophoretic separation on the agarose gel (Figure 4A).

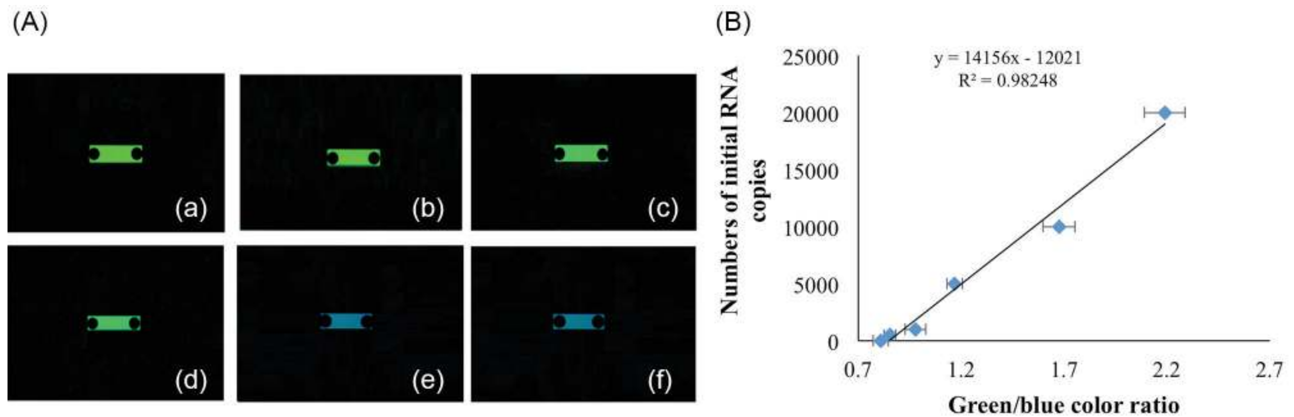

Figure 4. (A) Visual detection performed on-chip: (a) 20000 copies, (b) 10000 copies, (c) 5000 copies, (d) 1000 copies, (e) 500 copies of RNA from serum sample of infected patients, (f) negative control (serum sample of healthy patient); (B) digital analysis of the microchannel images by ImageJ software[59] for semi-quantitative correlation of green/blue ratio fluorescence intensity (n = 6).

The fluorescence analysis is considered useful for fast and simple detection methods. The use of fluorescent intercalating dye allows for the quantification of the amplicon product in real-time[60-63] or by image data analysis.[41,49] Here, the image analysis allowed a rapid semi-analytical quantification. The images were evaluated using ImageJ[59] program. First, the intensity of color was measured using the green channel of the RGB (red, green, blue) color channels. Figure S1 (Supplementary Information section) shows a slightly linear behavior, with coefficient of determination (R^2) = 0.9574, of the green fluorescence intensity *vs*. the logarithm of the number of initial copies of DNA. In order to improve the linearity and sensitivity of the data, we change the intensity of the green color by the green/blue ratio, also using the RGB color channels. Figure 4B shows the reasonable linear behavior, with R^2 = 0.9825, of the logarithm of the number of initial copies of DNA *vs*. green/blue ratio. Furthermore, this change caused a significant improvement in sensitivity.

Visual detection has been shown to be an excellent alternative to gel electrophoresis to obtain fast results, reducing the time detection from 60 to 2 min.

Conclusions

The RT-LAMP described in this study is a simple one-step method for rapid molecular diagnostics of dengue virus using a disposable microdevice. The reaction in the PeT microsystem proved to be an inexpensive and accurate method that allowed for a rapid genome detection of dengue virus.

In the assays carried out in the PeT microchip, it was possible to detect amplicons in reactions that started with 0.8 fg μL^{-1} of RNA or 200 copies μL^{-1} of RNA both for off-chip detection (by gel electrophoresis) and for on-chip detection (visual detection). The advantage of visual detection provided by LAMP is to obtain fast results, eliminating the need for electrophoresis.

We showed that using an RNA target, Bst 3.0, we achieved the highest level of reverse transcriptase activity reducing the time for final results and can perform single-enzyme RT-LAMP for DENV detection in serum samples.

A rapid test capable of confirming the early infection of dengue can avoid expensive and time-consuming tests as the conventional molecular methods. The conventional molecular diagnostics of dengue involves RT-PCR followed by semi-nested PCR. This method requires pure RNA, and thus a preliminary step in RNA purification must be performed to remove inhibitory substances (e.g., proteins) present in clinical samples. In contrast, RT-LAMP is not significantly affected by inhibitors and here we showed that it can be performed directly in a complex sample, eliminating the RNA purification step. Therefore, while a conventional methodology of molecular diagnosis for dengue would take at least 300 min (including electrophoresis), we showed in this study that we could carry out a molecular diagnosis of dengue in 17 min (including visual detection) using on average only 0.2 µL of serum from patients infected with dengue virus. Besides that, the cost of our method is much lower than the cost of a PCR-based test. While a diagnostic involving PCR methodology costs on average ca. US$ 80, a test using our method and our device costs less than US$ 1 (including microchip and reagents). Taking into consideration the instrumentation required for both tests, the conventional PCR instrumentation costs more than US$ 5,000, while a simple thermoblock costs less than a US$ 1,000.

Performing a RT-LAMP reaction in a disposable and low-cost microchip is a first step in the application of molecular diagnostics at the point of care. The entire system

can be miniaturized to have a specific and simple molecular diagnosis that can be taken to a remote location. Due to its simple operation, because it is performed in a single step, and it dismisses the need for sophisticated instrumentation, the RT-LAMP performed in PeT microchip has proven to be a valuable tool for molecular diagnosis of dengue, presenting a great potential for point care applications for both diagnostics and epidemiological studies, especially in developing countries.

Supplementary Information

Supplementary information is available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

CNPq (grant No. 422016/2016-0, 407666/2016), FAPEG (grant No. 201410267001777), INCTBio (FAPESP grant No. 2014/50867-3 and CNPq grant No. 465389/2014-7), Instituto Nacional de Ciência e Tecnologia (INCT) de Estratégias de Interação Patógeno Hospedeiro and FAPESP (grant No. 2015/17145-7) are kindly acknowledged for the grants.

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Submitted: January 3, 2019
Published online: May 14, 2019

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