Editorial

QUASR RT-LAMP: a potential technology for development of diagnostics for point of care settings

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The threat of emerging or re-emerging arboviral diseases are increasing, due to a number of factors including global change in environment, rapid and unplanned urbanization, indiscriminate use of chemicals for vector control, rapid evolution of the viral pathogen and their geographic expansion etc. (1,2). With the advancement of the travel and transport, diseases emerging or re-emerging in one geographical region are rapidly spread to other regions (3). During the last decade, a number of viral diseases have emerged and spread globally, including many arboviral diseases that have re-emerged and caused havoc in different parts of the world (4-7). This includes a long list comprising of Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome Coronavirus (MERS), Ebola, Nipah, Avian flu, Swine flu, Dengue (DENV), Chikungunya (CHIKV), Zika (ZIKV) etc. In addition to the naturally emerging or re-emerging pathogens, the risk of deliberate use of pathogens for malicious intentions have also increased in the recent times with the advancement of genetic manipulation techniques (8,9). Nevertheless, the key challenge in control and containment of these diseases is their early detection at the point-of-care (POC) or in field conditions, in a resource poor setting (10). To qualify for a POC, an integrated diagnostic method should incorporate (I) a sensitive and specific amplification reaction (should be less sensitive to sample impurities), (II) a low power requiring reaction module and (III) a sensitive detection technique, allowing visualization of the results thorough naked eye or simple optical device.

At present available diagnostic technologies are primarily aimed at detection of pathogen antigens or the pathogen nucleic acids (DNA or RNA). Most of these techniques are based on different variants of antigen-antibody based assays (e.g., ELISA, immunochromatographic strips, dot blot etc.) or nucleic acid amplification methods (PCR, RT-PCR, qPCR, etc.) (11-14). These technologies are widely used in laboratory based diagnosis, but have several limitations that preclude their adoption for field conditions or POC settings. PCR amplification based techniques are highly sensitive and specific, but the requirement of highly pure nucleic acid preparations, power intensive thermal cycler devices as well as sophisticated post-PCR analysis equipments limit their applications outside laboratory conditions. In comparison, antigen-antibody based detection do accommodate certain levels of flexibility in allowing crude samples for analyses, but there are certain issues regarding sensitivity and cross reactivity, especially in case of viral antigens having a high rate of evolution. Nonetheless, as compared to peptide based methods, nucleic acid based methods are relatively easy to design, perform, allow certain degrees of variation in the primer sequences through use of degenerate primers and most importantly being amplification based, they are capable of detecting clinically relevant amounts of the target templates in the sample.

Considering these advantages of nucleic acid based techniques, efforts were made towards inventing techniques that do not require programmable thermal cycler devices, and to develop detection techniques for visual interpretation of results. As a result of these efforts a number of isothermal techniques (LAMP, NASBA, TSA, HDA, RPA etc.) were developed that amplify nucleic acids at a single temperature and thereby allow reaction to

occur in simple uniform heating devices (15,16). Among the isothermal techniques, LAMP has gained considerable popularity over other techniques, owing to its definite advantages. This method uses 4-6 primers at a constant temperature of 60–65 ℃, which gives high specificity to the reaction. Sensitivity of LAMP is <10 copies per reaction, which is comparable to conventional PCR, while 'time to positive' is lesser than PCR. Furthermore, LAMP supports various chemistries for detection of the amplification thorough fluorescence, turbidity or colorimetry (11,17,18). Additionally, LAMP based amplification assay have been reported to tolerate template impurities to certain extent (as compared to other methods), making it very useful diagnostic method for POC settings (19). Earlier, the potential of the LAMP was restricted due to its inability to accommodate multiplexing, but recently quencher-fluorophore chemistries have enabled LAMP assays with multiplexing capabilities, further widening its reliable application in diagnostics (20). Finally, the revolutions in mobile phone technologies have resulted in availability of affordable, extremely powerful hand-held smartphones that can simultaneously works as a hand-held temperature controller for reaction module, as well as can detect, process, and communicate signals.

Recently, Priye and colleagues (21) developed an integrated smartphone based platform and demonstrated its efficacy in rapid detection of three very important arboviral diseases. In this work, the researchers integrated a multiplex Reverse-Transcriptase-LAMP (RT-LAMP) with a QUASR (Quenching of Unincorporated Amplification Signal Reporters) based detection chemistry. The authors utilized a smartphone and application software for control of the LAMP reaction module as well as for the acquisition and processing of the QUASR signals. This system hallmarks the development of simple, extremely portable, low cost and very low power requiring diagnostic platforms, best suited for resource poor settings. Initially the researchers optimized primers designed from different highly conserved regions of the viral genome (Env, NS1, NS3, NS5 & 3'-UTR) and determined the best primer pair on the basis of sensitivity, speed and accuracy of detection. The best primer pair (targeting NS5) was found to be highly sensitive, having the ability to detect even ten copies of the RNA genome per reaction. Subsequently, the researchers tested the QUASR detection chemistry by specific labelling of the 5' end of the BIP primer with Cy5 along with a short complimentary quencher probe having a 3' Iowa Black RQ quencher. With this arrangement of the primers, reporter and quenchers, RT-LAMP reactions with ZIKV amplicons resulted in bright fluorescence on being excited by appropriate light source. On the other hand negative reactions remained dark (no fluorescence). Furthermore, the LAMP assay was not compromised with incorporation of the Cy5 and also allowed simultaneous real time monitoring of the amplification with a DNA intercalating dye (SYTO 9). In this study the researchers also attempted to see if the RT-LAMP assay could be used with intact pathogens and found that the assay to be comparably efficient in detecting intact viruses. Finally the researchers integrated their QUASR-RT-LAMP with a simple device incorporating three components, namely a heating module for LAMP reaction, a reaction housing module and signal detection and processing. The detector comprised of a LED and multi-pass band filter based excitation source which was connected to a Smartphone application "*LAMPtoGo*" through wireless Bluetooth connection. The QUASR signals were detected by the CMOS based smartphone camera followed by its analysis through the application software.

Apart from designing an '*all-in-one*' approach towards diagnosis of three clinically important arboviruses, the present study also reported other factors, which are also considerably important in developing a POC diagnostic assay. The researchers attempted to stabilize the reagents for shelf stable ready preparations of the QUASR-RT-LAMP assay by drying the reagents along with a commercially available stabilization mixture. The dry stabilized reagent mixes were found to be comparably efficient as fresh reagents and yielded detectable signal by real time as well as end-point QUASR. The reagents were stable at temperatures up to 40 ℃ for minimum one month. The researchers also studied the effects of virus spiked human clinical samples on QUASR-RT-LAMP assay. Although 'time to positive' was increased in presence of crude sample matrix but the results of QUASR end point detection was confirmatory, even at very low concentration of 10^3 PFU/mL. Although the intensity of fluorescence decreased with lower concentrations of the virus in blood and saliva matrix, the results of QUASR between positive and negative samples were found to be conclusive.

Taken together, the findings of important work paper illuminate the significance of integrating different technologies and their optimization for meeting the requirements of a POC diagnostic. The multiplex QUASR RT-LAMP may prove to be a promising approach for

detection number of a number of pathogens of public health importance as well as military significance. The article by Priye and colleagues will motivate other researchers working in related areas to use and develop this technology for new POC diagnostics, relevant to their geographical regions.

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