Smart Phone-enabled Diagnostic Platform For Detection of Pathogen Nucleic Acids

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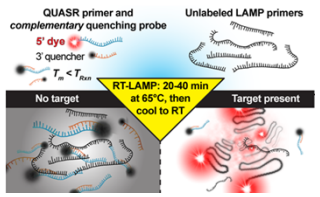
Abstract: Nucleic acid amplification tests (NAATs) like real-time PCR offer outstanding sensitivity and sensitivity, but generally require a well-equipped laboratory and trained staff to carry out sample prep and complex protocols. Isothermal protocols like LAMP can be simpler than PCR, but have other drawbacks such as a high rate of false positives. To enable simpler, point-of-care testing, we demonstrate the following innovations:

1) An improved approach to monitoring LAMP and RT-LAMP reactions using a closed tube, target-specific chemistry, with bright endpoints and capability for multiplexed detection,
2) A novel self-sealing microfluidic device containing dried-down assay reagents that simplifies LAMP in point-of-care settings, and
3) A smart phone-enabled assay platform that automates assay operation, interpretation, and reporting of data.

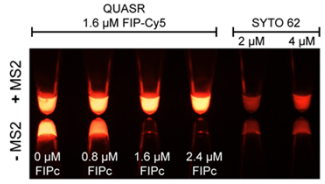
We demonstrate this suite of technologies for viral pathogens including West Nile virus, chikungunya virus, and Ebola virus, as well as *Plasmodium falciparum*.

Quenching of Unamplified Signal Reporters (QUASR) RT-LAMP: We have developed a patent-pending approach to generating bright, target-specific fluorescent endpoints in LAMP and RT-LAMP, based upon interaction between a fluorescently labeled primer and a short quench probe. This approach improves upon non-specific indicators of DNA synthesis such as intercalating dyes, quenched calcein, turbidity, or color change (*e.g.* hyrdoxynaphthol blue).

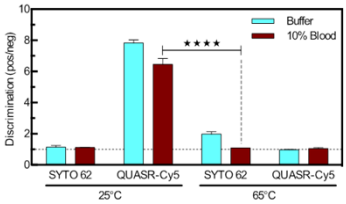
A. Principle of QUASR LAMP



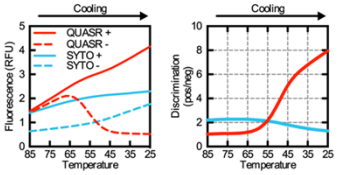
B. Demonstration of endpoint quenching with QUASR *vs* intercalating dye SYTO 62



C. QUASR RT-LAMP improves signal in "direct" LAMP from whole blood.

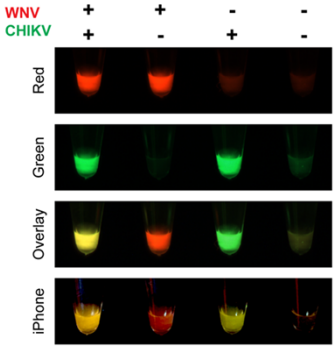


QUASR fluorescence is diminished much less than SYTO 62 with 10% whole blood.



The QUASR endpoint signal "develops" as the reaction cools to ambient temperature, without opening the tube.

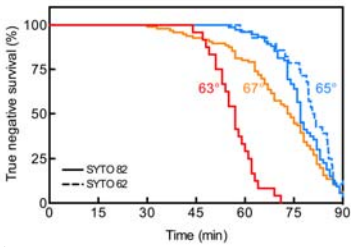
D. Duplex detection of West Nile virus (WNV) and chikungunya virus (CHIKV)



The "Red" and "Green" images were acquired with a machine vision camera, using LED excitation and hard-coated glass filters for fluorescence. The "iPhone" image was taken using a blue LED flashlight for excitation, through an amber colored plastic filter, using a white balance card set to adjust exposure.

E. Reduction of false positives in WNV RT-LAMP using QUASR *vs* SYTO dyes

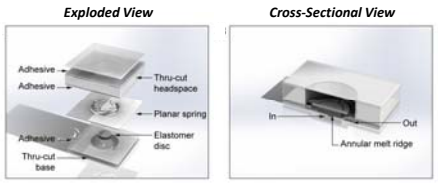
Reporter Mixture	Decision fluorophore	False Positives X/Y (%)
SYTO only	SYTO 62	25/28 (89%)
SYTO + QUASR	SYTO 62	42/117 (36%)
	QUASR (all)	0/117 (0%)
QUASR only	QUASR (all)	1/80 (1%)*



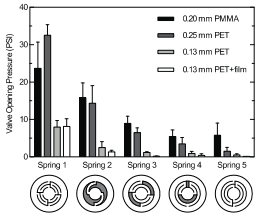
Most reactions monitored "real time" with SYTO only eventually generate false-positives. When these same reactions are monitored by QUASR in a separate channel, they remain dark (negative).

Ball *et al*, 2016 (manuscript in review)

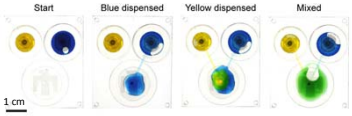
Microfluidic devices for one-step QUASR LAMP: Previously reported microfluidic devices developed for high-temperature incubations such as LAMP require an active sealing step, for example by applying tape or actuating a valve. We have developed a novel planar check valve that allows fluid to be added to a channel containing dried reagents under pressure, and then seals against backflow or evaporation. The valve further enables staged delivery of fluids, or creation of simple finger-powered pumps.



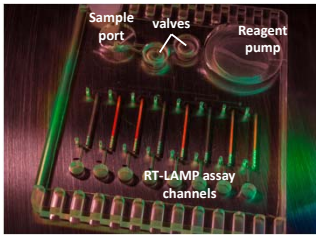
Valves can be constructed by rapid prototyping techniques as well as machining or molding



Spring pattern and material determine opening pressure

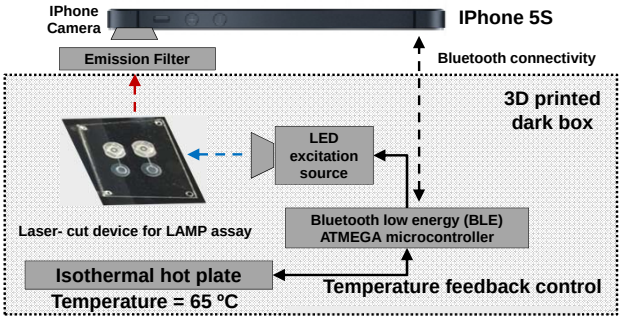


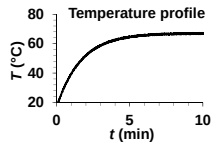
Valves enable staged reagent delivery in a finger-actuated device

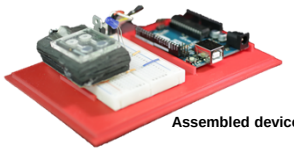


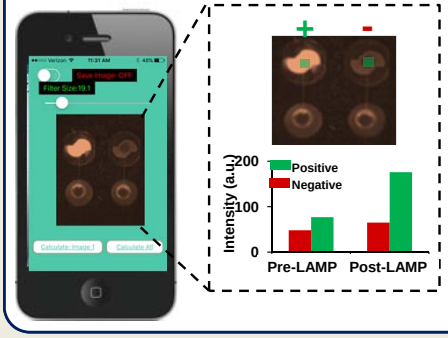
Two valves enable staged sample dilution (with onboard buffer) and dead-end delivery to array of channels with dried QUASR LAMP reagents.

Integrated smartphone detection unit: We utilize the built-in imaging, data processing, and wireless networking capabilities of a ubiquitous smart phone – in this case an older model iPhone 5S is inexpensive yet sufficiently powerful to support our assay.









We have developed a *Portable LAMP* app for Android and iPhone.

Focus and exposure are locked for the duration of an experiment.

Average, maximum, and minimum RGBA pixel values for each region of interest can be plotted or tabulated on screen.

We have also developed an app to send data (via iPhone) to Amazon cloud.

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