PCR*opsis*™ Reagent RVD-RT Facilitates Visual Detection of SARS-CoV-2 in Transport Medium using RT-LAMP and May Improve Sensitivity

Francis Buan Hong Lim[1], Abhignyan Nagesetti[1], Kevin Moreno[1], Agustin Galecio[1], Ian Cheong[1,2,3,4], Obdulio Piloto[1]

Affiliations:
[1] Entopsis, Inc., USA
[2] Temasek Life Sciences Laboratory, Singapore
[3] Department of Biological Sciences, National University of Singapore, Singapore
[4] Pathnova Laboratories Pte Ltd, Singapore

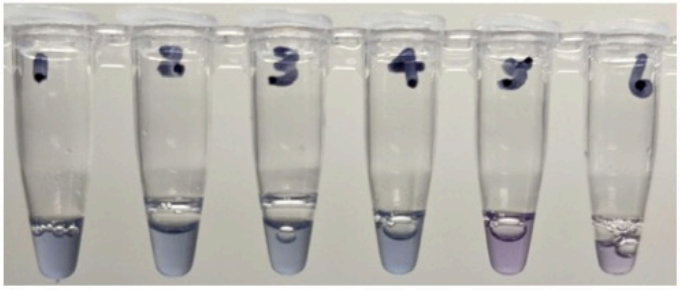
Correspondence to: info@entopsis.com

Abstract:
Isothermal PCR offers fast and visually detectable diagnostic results without the need for specialized equipment, like a thermal cycler. This approach is ideal for point of care testing, testing in low resource settings, and population testing when fast turn around times are critical. However, isothermal PCR requires isolated RNA or DNA as the starting test material. RNA and DNA isolation requires various laboratory equipments, such as centrifuges, vacuum adapters, or vortexers, along with a variety of buffers and reagents. In this report, we describe a simple method for performing reverse transcription loop-mediated isothermal amplification (RT-LAMP) without nucleic acid extraction. SARS-CoV-2, a RNA virus, in transport medium and PCR*opsis*™ Reagent RVD-RT, a room temperature Next Generation Direct PCR™ product, were used for this proof of concept study. The study concluded that Reagent RVD-RT mediates detection of 39 viral copies / µL in transport medium, translating to 3.9 viral copies / µL in the 25 µL RT-LAMP reaction mixture. This detection limit is an improvement of ~6-fold compared to the reported sensitivity of 25 viral copies / µL by the test manufacturer. As such, PCR*opsis*™ Reagent RVD-RT can be used in combination with RT-LAMP kits, resulting in comparable or potentially better sensitivity compared to isolated viral RNA.

Results:

a) Serial Dilution

39K	3900	390	39	3.9	0.39	viral copies / µL in sample
3900	390	39	3.9	0.39	0.039	viral copies / µL in RT-LAMP



b) Controls

Reagent RVD-RT	Reagent RVD-RT	Isolated viral RNA	RNA Control from Kit	Water	Transport Medium	
39	39	>100	125	0	0	viral copies / µL in sample
3.9	3.9	>20	25	0	0	viral copies / µL in RT-LAMP

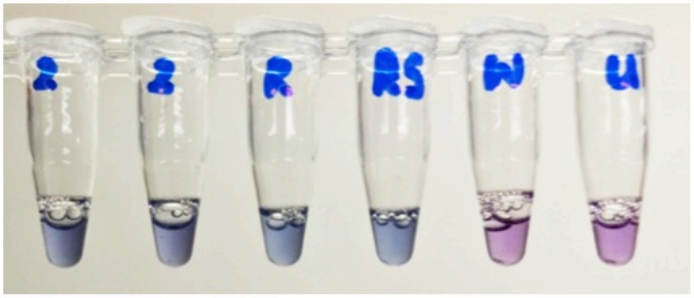


Figure 1. Reagent RVD-RT + RT-LAMP allows for the visual detection of 39 viral copies / µL SARS-CoV-2 spiked into transport medium. (A) Visual detection of serially diluted SARS-CoV-2 by Reagent RVD-RT + RT-LAMP at 60˚C. (B) Examples of positive controls (isolated SARS-CoV-2 RNA) and negative controls (water and transport medium) showing a visible color difference. All samples were tested twice. Representative results shown.

Key Conclusions:

- PCR*opsis*™ Reagent RVD-RT + RT-LAMP allows for visual detection of 39 viral copies / µL of SARS-CoV-2 in transport medium in 30 minutes at 60˚C without the need for a thermal cycler.
    - A starting sample containing 39 viral copies / µL results in 3.9 viral copies / µL in the RT-LAMP reaction tube
        - Mix sample 1:1 with Reagent RVD-RT: 39 / 2 = 19.5 viral copies / µL

        - Add 5 µL of processed sample to 20 µL RT-LAMP reaction: 19.5 * 5 = 97.5 / 25 = **3.9 viral copies / µL**
    - The manufacturer reported sensitivity of the SARS-CoV-2 RT-LAMP kit is 25 viral copies / µL in the RT-LAMP reaction mixture

- Further studies focused on the extent PCR*opsis*™ Reagent RVD-RT improves the sensitivity of RT-LAMP are warranted give observation from this study. We hypothesize that Reagent RVD-RT may improve the amplification efficiency of RT-LAMP by making amplification gene sites more accessible to enzymes.

Methods:

Materials:
- ATCC: 2019 Novel Coronavirus (VR-1986HK)
- BD™ Universal Viral Transport (UVT)
- Entopsis: PCR*opsis*™ Reagent RVD-RT
- RayBiotech: COVID-19 Rapid Isothermal PCR Kit
- Stellar Scientific: Thin-walled PCR tubes
- Chai: Open qPCR Thermocycler
- Qiagen: QIAamp Viral RNA Kit

Prepare diluted viral samples in transport medium:
1. Perform 1 : 10 serial dilutions of SARS-CoV-2 in transport medium
2. Mix thoroughly

PCR*opsis*™ Reagent RVD-RT + RT-LAMP:
1. Thoroughly mix 20 µL of reagent with 20 µL of diluted viral samples in a thin walled tube (0.2 mL) and cap tube
2. Incubate the reagent mixture with viral sample for 10 minutes at 25˚C and mix thoroughly
3. Add 5 µL of processed sample to 20 µL of RT-LAMP mix
    a. RT-LAMP mixture:
        i. Master Mix: 15 µL
        ii. SARS-CoV-2 primer mix: 5 µl
        iii. Processed sample: 5 µL
4. Incubate samples at 60˚C for 30 minutes in a heating block
5. Observe color change

Viral RNA extraction:
Follow manufacturer's protocol: Qiagen QIAamp Viral RNA Kit