PCR*opsis*™ Reagent RVD-RT enables detection of less than 50 cfu / mL of gram positive and negative bacteria at room temperature.

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:
Extraction-free PCR at room temperature makes PCR testing easily integrated into laboratory automation, thus saving time and money while minimizing sample testing backlogs. However, many room temperature direct PCR products on the market fail to sufficiently lyse bacteria and present genomic DNA for amplification in high sensitivity assays. In this report, we show that PCR*opsis*™ Reagent RVD-RT enables extraction free direct PCR at room temperature for both gram positive and negative bacteria. The reagent was able to process bacterial samples containing as little as 24 and 32 cfu / mL. As such, PCR*opsis*™ Reagent RVD-RT can be used as an extraction-free approach to amplify bacteria from specimens in transport medium.

Results:

Staphylococcus aureus		Pseudomonas aeruginosa	
cfu / mL	Ct	**cfu / mL**	Ct
240,000	22.2	**320,000**	20.81
24,000	25.03	**32,000**	24.83
2,400	26.83	**3,200**	29.15
240	30.55	**320**	30.16
24	33.46	**32**	33.68

Table 1. Reagent RVD-RT mediates direct PCR amplification of samples with less than 50 cfu / mL of S. aureus and P. aeruginosa. The use of Reagent RVD-RT as an alternative to DNA extraction for PCR-mediated detection of diluted bacteria in transport medium resulted in Ct values ranging from 20 ~ 34. Each sample was tested in duplicates and the indicated Ct is the average of two readings.

Key Conclusions:

- PCR*opsis*™ Reagent RVD-RT mediates detection of gram positive and negative bacteria in transport medium at concentrations less than 50 cfu / mL
- PCR*opsis*™ Reagent RVD-RT facilitates direct PCR at room temperature, indicating that the reagent is able to lyse tested bacteria and make genomic DNA accessible to PCR without heating

Methods:

Materials:

- Microorganisms (ATCC): Staphylococcus aureus, Pseudomonas aeruginosa
- BD™ Universal Viral Transport (UVT)
- IDT: DNA primers & probes (see Table 1)
- Entopsis:
  - PCR*opsis*™ Reagent RVD-RT
  - PCR*opsis*™ 5x PCR Master Mix
- Stellar Scientific: Thin-walled PCR tubes
- Chai: Open qPCR Thermocycler

Table 1. Primers and probes used for the detection of indicated microorganisms in universal transport medium

Microorganism	Primer	Probe	Sequence (5'-3')
Staphylococcus aureus	S868F		CCACATGCCTCTAATAATG
	S1064R		GCGATTTTATTTTCTTTTGTAC
		S1024P	ATGCCATGCCTCCAAATATCGC
Pseudomonas aeurginosa	Pa23F		TCCAAGTTTAAGGTGGTAGGCTG
	Pa23R		CTTTTCTTGGAAGCATGGCATC
		Pa23P	AGGTAAATCCGGGGTTTCAAGGCC

Prepare diluted bacterial samples in transport medium:

1. Perform 1:10 serial dilutions of bacteria in transport medium.
2. Mix thoroughly.
3. Plate bacterial dilutions to determine colony-forming units (cfu).

Studies with PCR*opsis*™ Reagent RVD-RT:

1. Thoroughly mix 20 µL of reagent with 20 µL of diluted bacterial samples in a thin walled tube (0.2 mL) and cap tube.
2. Incubate the reagent mixture with bacterial sample for 10 minutes at room temperature and mix thoroughly.
3. Add 5 µL of processed sample to 15 µL of PCR mixture.
    a. PCR mixture:
        i. PCR*opsis*™ PCR Master Mix, 5X: 4 µL,
        ii. IDT primer / probe: 1.5 µl,
        iii. Nuclease-Free Water: 9.5 µl,
        iv. Processed sample: 5 µL.
4. Run samples on PCR Thermocycler for 45 cycles.
    a. DNA Amplification: 95˚C;30 seconds, 55˚C;30 seconds, 72˚C;30 seconds for 45 cycles,
    b. Extension: 72˚C;60 seconds,
    c. Hold: 4˚C.

References:

1. Pan, Y. et. al. Viral load of SARS-CoV-2 in clinical samples. Lancet Infectious Diseases (2020)
2. Wang, W. et. al. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA (2020)
3. Zheng, S. et. al. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. BMJ (2020)
4. Hasanoglu, I. et. al. Higher viral loads in asymptomatic COVID-19 patients might be the invisible part of the iceberg. Infection (2020)