PCR*opsis*™ Reagent RVD Facilitates DNA Extraction-free Amplification of Bacterial Gene Targets

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

Affiliations:
[1] Entopsis LLC, USA
[2] Madison Core Laboratories, USA
[3] Temasek Life Sciences Laboratory, Singapore
[4] Department of Biological Sciences, National University of Singapore, Singapore.

Correspondence to: info@entopsis.com

Abstract:
Bacterial DNA extraction is unnecessary and presents burdensome steps towards the detection of desired microorganisms. Here we outline and validate a simple, extraction-free protocol that facilitates amplification of species-specific gene targets. This approach replaces DNA extraction with a 15-minute heating step utilizing PCR*opsis*™ Reagent RVD. This extraction-free protocol resulted in a limit of detection (LoD) less than 100 cfu / mL for gram positive and negative microorganisms.

Methods:

Materials:
- Microorganisms (ATCC): staphylococcus aureus, Pseudomonas aeruginosa
- PCR reagents (Bulldog Bio): 10x PCR Buffer, dNTPs, Taq polymerase (5U/µL), Nuclease-free water
- IDT: DNA primers & probes (see Table 1)
- BD™ Universal Viral Transport (UVT) medium
- Entopsis: PCR*opsis*™ Reagent RVD
- Stellar Scientific: Thin-walled qPCR tubes
- Chai: Open qPCR Thermocycler

Limit of Detection (LoD) Study:
1. Prepare 1:10 serial dilutions of microorganisms in BD™ UVT.
2. Store serially diluted samples at 4˚C for 24 hours so as to mimic overnight shipping of clinical samples.
3. Thoroughly mix 50 µL Reagent RVD with 50 µL of diluted samples in a thin walled tube (0.2 ~ 0.6 mL) and cap tubes.
4. Heat mixture of Reagent RVD / microbial sample for 15 minutes at 95˚C and let cool at room temperature for ~10 seconds before continuing.
5. Mix thoroughly.

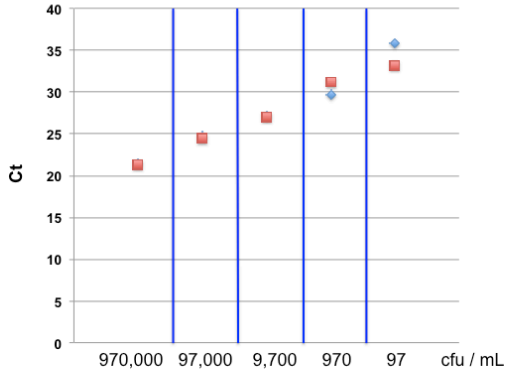
6. Add 5 µL of heated Reagent RVD / bacterial sample to 15 µL of qPCR mixture.
    a. qPCR mixture:
        i. 10x PCR Buffer: 2 µl
        ii. Taq Polymerase (5U/µl): 0.4 µl
        iii. 40 mM dNTPs 0.1 µl
        iv. Forward primer (10 pmol/ µl): 1 µl
        v. Reverse primer (10 pmol/ µl): 1 µl
        vi. Probe (10 pmol/ µl): 0.5 µl
        vii. Nuclease-Free Water: 10 µl
7. Run samples on qPCR Thermocycler for 45 cycles.
    a. DNA Amplification:
        i. 95˚C 5 minutes (initial denaturation)
        ii. 95˚C 30 seconds
        iii. 55˚C 30 seconds
        iv. 72˚C 30 seconds
        v. 72˚C 60 seconds (final extension)
        vi. 4˚C hold

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

Results:

A) Serial dilution: S. aureus



B) Replicates at LoD: S. aureus

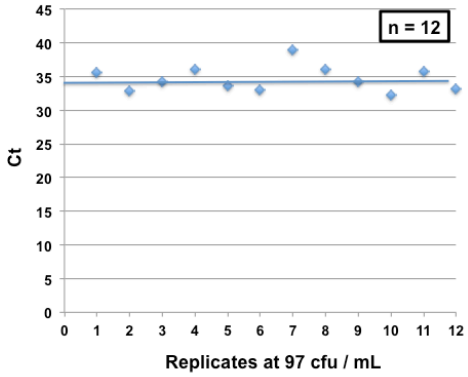
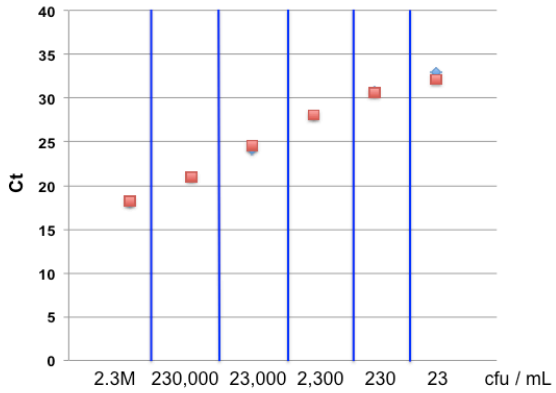


Figure 1. Reagent RVD facilitates extraction-free detection of S. aureus in universal transport medium with a LoD <100 cfu / mL. (A) Gene fragment amplification was reliably observed at microbial concentrations of 970,000 – 97 cfu / mL with Ct values of 22~36 when duplicate samples were tested. (B) All 12 replicate samples at 97 cfu / mL resulted in amplification.

A) Serial dilution: P. aeruginosa



B) Replicates at LoD: P. aeruginosa

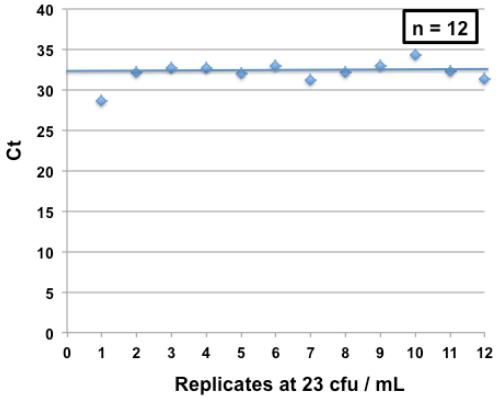


Figure 2. Reagent RVD facilitates extraction-free detection of P. aeruginosa in universal transport medium with a LoD <100 cfu / mL. (A) Gene fragment amplification was reliably observed at microbial concentrations of 2,300,000 – 23 cfu / mL with Ct values of 18~34 when duplicate samples were tested. (B) All 12 replicate samples at 23 cfu / mL resulted in amplification.

Conclusions & Discussion:

- Reagent RVD combined with qPCR was able to reliably detect Staphylococcus aureus and Pseudomonas aeruginosa in universal viral transport medium with a limit of detection <100 cfu / mL (97 and 23 cfu / mL, respectively). This is on par with peer-reviewed reports[1,2,3].
- The data supports the replacement of traditional DNA extraction with Reagent RVD for the detection of bacteria in universal transport mediums.
- Users should perform pilot studies to optimize the heating step when using Reagent RVD with difficult to lyse bacteria or when suboptimal lysis is observed. A 20-minute, instead of a 15-minute, heating step should be considered in these situations.

References

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