

PCRopsis™ Reagent RVD with RVD Enhancer Facilitates DNA Extraction-free Amplification of Bacterial Gene Targets

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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 PCRopsis™ Reagent RVD with RVD Enhancer. 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
- IDT: DNA primers & probes (see Table 1)
- BD™ Universal Viral Transport (UVT) medium
- Entopsis:
 - PCRopsis™ Reagent RVD with RVD Enhancer
 - PCRopsis™ 5x PCR Master Mix
- 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 RVD Enhancer with 50 µL of diluted samples in a thin walled tube (0.2 ~ 0.6 mL) and cap tubes.
4. Heat mixture of reagent / 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 / bacterial sample to 15 µL of qPCR mixture.

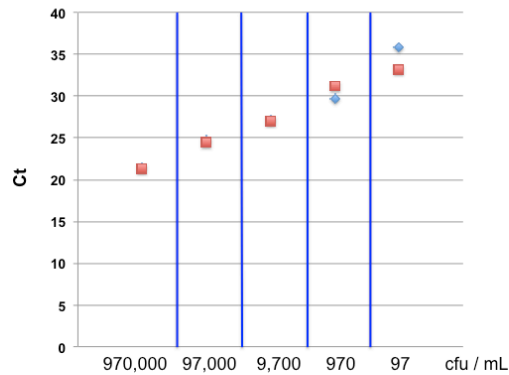
- a. qPCR mixture:
 - i. PCR_{opsis}[™] 5x PCR Master Mix: 4 µl
 - ii. Forward primer (10 pmol/ µl): 1 µl
 - iii. Reverse primer (10 pmol/ µl): 1 µl
 - iv. Probe (10 pmol/ µl): 0.5 µl
 - v. Nuclease-Free Water: 8.5 µl
7. Run samples on qPCR Thermocycler for 45 cycles.
 - a. DNA Amplification:
 - i. 95°C 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 aeruginosa	Pa23F		TCCAAGTTTAAGGTGGTAGGCTG
	Pa23R		CTTTTCTTGGAAGCATGGCATC
		Pa23P	AGGTAAATCCGGGGTTTCAAGGCC

Results:

A) Serial dilution: *S. aureus*



B) Replicates at LoD: *S. aureus*

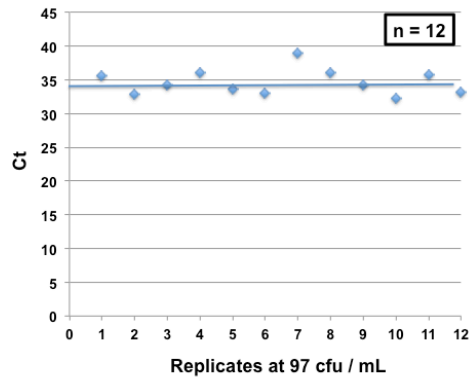
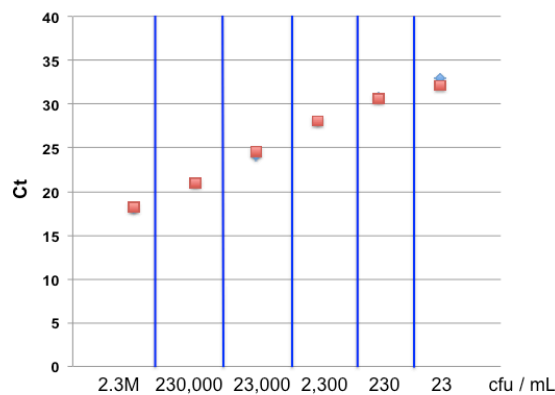


Figure 1. Reagent RVD with RVD Enhancer facilitates extraction-free detection of *S. aureus* in universal transport medium with a LoD <100 cfu / mL. (A) Gene fragment amplification was reliably observed at microbial concentrations of 970,000 – 97 cfu / mL with Ct values of 22~36 when duplicate samples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

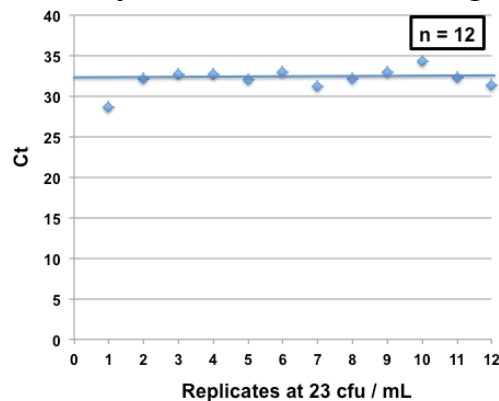


Figure 2. Reagent RVD with RVD Enhancer facilitates extraction-free detection of *P. aeruginosa* in universal transport medium with a LoD <100 cfu / mL. (A) Gene fragment amplification was reliably observed at microbial concentrations of 2,300,000 – 23 cfu / mL with Ct values of 18~34 when duplicate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

Conclusions & Discussion:

- Reagent RVD with RVD Enhancer 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 with RVD Enhancer for the detection of bacteria in universal transport mediums.
- Users should perform pilot studies to optimize the heating step when using Reagent RVD with RVD Enhancer 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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2. Bono, J. L. *et al.* Evaluation of a Real-Time PCR Kit for Detecting *Escherichia coli* O157 in Bovine Fecal Samples. *Appl. Environ. Microbiol.* 70, 1855–1857 (2004).
3. Trnčíková, T., Hrušková, V., Oravcová, K., Pangallo, D. & Kaclíková, E. Rapid and Sensitive Detection of *Staphylococcus aureus* in Food Using Selective Enrichment and Real-Time PCR Targeting a New Gene Marker. *Food Analytical Methods* 2, 241–250 (2009).