PCR*opsis*™ Support and Lysis Beads Offer Improved Detection of Difficult to Lyse Microorganisms

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Abstract:
The detection of fungi and other microorganisms involves a time-consuming and cumbersome process of extracting DNA before PCR amplification. These issues delay results and increase the costs of such tests. In this report, we present a simple, low-cost, and robust means of amplifying DNA from low concentrations of yeast, gram-positive bacteria, and gram-negative bacteria spiked into urine. This alternative nucleic acid extraction method allows for the detection of less than 50 colony forming units (CFU) / mL of fungi and bacteria, with an extraction time of around 20 minutes and no specialized equipment. The simplicity of this approach lends itself well to automation.

Results:

C. albicans

CFU / mL	Reagent RVD with RVD Enhancer			
	0 uL	2 uL	20 uL	Support
8.3 Million	19.4	19.84	19.11	
830,000	20.88	20.22	21.45	
83,000	25.17	25.72	24.13	
8300	28.91	28.76	28.54	
830	32.08	32.76	32.63	
83	34.84	34.63	35.24	
8.3	38.24	37.66	37.03	

P. aeruginosa

CFU / mL	Reagent RVD with RVD Enhancer			
	0 uL	2 uL	20 uL	Support
4.5 Million	18.77	18.51	17.69	
450,000	20.52	20.24	20.04	
45,000	24.51	24.33	23.88	
4500	26.46	26.76	25.97	
450	30.31	29.46	30.09	
45	33.37	33.11	33.49	
4.5	38.01	37.33	37.68	

S. aureus

CFU / mL	Reagent RVD with RVD Enhancer			
	0 uL	2 uL	20 uL	Support
3.2 Million	30.64	18.68	19.04	
320,000	34.56	20.33	20.51	
32,000	0	24.32	23.66	
3200	0	27.65	27.27	
320	0	30.05	30.41	
32	0	34.52	34.84	
3.2	0	0	0	

Figure 1. The addition of Support to Reagent RVD with RVD Enhancer helps in the amplification of target genes from select microorganisms. The addition of Support to Reagent RVD with RVD Enhancer improves the detection of gram-positive Staphylococcus aureus, and has no effect on gram-negative Pseudomonas aeruginosa or yeast, Candida albicans. Each sample was tested in duplicates and the indicated Ct is the average of two readings.

		Reagent Added First to Tube	Sample Added First to Tube	
S. aureus	**CFU / mL**	**Reagent RVD with RVD Enhancer**		
		0 uL	**2 uL**	**2 uL** **Support**
	3.2 Million	30.64	18.91	30.29
	320,000	34.56	20.64	35.28
	32,000	0	25.21	0
	3200	0	27.08	0
	320	0	30.66	0
	32	0	34.25	0
	3.2	0	0	0

Figure 2. Reagent RVD with RVD Enhancer + Support should be added to the test tube first, before addition of the urine test sample. The direct PCR reagent should be added to the test tube first for optimal direct PCR. Each sample was tested in duplicates and the indicated Ct is the average of two readings.

	CFU / mL	PCRopsis Lysis Beads	Generic Lysis Beads
C. albicans	**6.7 Million**	18.95	22.72
	6700	28.39	33.56
	67	34.11	0
P. aeruginosa	**7.7 Million**	17.52	17.2
	7700	24.67	25.19
	77	33.31	34.96
S. aureus	**3.6 Million**	19.69	24.76
	3600	27.51	34.16
	36	34.33	0

Figure 3. PCR*opsis*™ Lysis Beads offer improved detection of select microorganisms compared to generic lysis beads. The coated PCR*opsis*™ Lysis Beads result in improved cellular lysis and minimal lost of target DNA from test samples. All samples were processed with Reagent RVD with RVD Enhancer + 2 µL / mL Support. Each sample was tested in duplicates and the indicated Ct is the average of two readings.

Key Conclusions:

- PCR*opsis*™ Reagent RVD with RVD Enhancer + Support amplify gene targets from P. aeruginosa and C. albicans when present at less than 10 CFU / mL in urine and S. aureus when present at less than 50 CFU / mL in urine.

- The addition of Support to Reagent RVD with RVD Enhancer is critical for low level detection of S. aureus in urine, but not for the detection of P. aeruginosa and C. albicans

- The addition of extra Support, beyond the recommended 2 µL / mL of Reagent RVD with RVD Enhancer does not offer an advantage under stated test conditions. A higher concentration of Support may be beneficial with other sample types or microorganisms

- Reagent RVD with RVD Enhancer + Support should be added to the test tube before addition of the test sample for optimal processing. This observation suggests that a percentage of target DNA binds to the surface of the tube and thereby is unavailable for amplification

- PCR*opsis*™ Lysis Beads offer improved lysis of C. albicans and S. aureus compared to alternative lysis beads. No improvement in the detection of P. aeruginosa was observed with PCR*opsis*™ Lysis Beads compared to generic lysis beads

Methods:

Materials:

- Microorganisms (ATCC): Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans
- IDT: DNA primers & probes
- Entopsis:
    - PCR*opsis*™ Reagent RVD with RVD Enhancer
    - PCR*opsis*™ Support
    - PCR*opsis*™ Lysis Beads
    - PCR*opsis*™ 5x PCR Master Mix
- Stellar Scientific: Thin-walled PCR tubes
- Chai: Open qPCR Thermocycler

Prepare diluted microorganisms in urine:

1. Perform serial dilutions of bacteria and yeast in human urine
2. Mix thoroughly
3. Plate bacterial dilutions to determine colony-forming units (cfu)

Pelleting microorganisms:

1. Centrifuge urine at >1,400xg for 10 minutes to pellet cells
2. Remove supernatant and leave ~250 µL of residual urine
3. Resuspend cell pellet using residual urine

Process bacteria and yeast with beads:

1. Add 200 µL of urine sample to a vial containing ~0.3 grams of PCRopsis™ Lysis Beads or generic lysis beads
2. Cap tube
3. Vortex on high for ~5 minutes

Process urine sample with direct PCR reagent:

1. Add 2 µL or 20 µL of PCR*opsis*™ Support to 1 mL PCR*opsis*™ Reagent RVD with RVD Enhancer, and mix thoroughly
2. Thoroughly mix 20 µL of freshly prepared reagent with 20 µL of lysis bead-processed urine sample in a thin walled tube (0.2 mL) and cap tube
3. Incubate the reagent mixture with microbial sample for 10 minutes at 95˚C and let cool at room temperature for ~10 seconds before continuing
4. Add 5 µL of processed sample to 15 µL of qPCR 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.
5. Run samples on PCR Thermocycler for 45 cycles.
    a. DNA Amplification:
        i. 95˚C 5 minutes (initial denaturation)
            1. 95˚C 30 seconds
            2. 55˚C 30 seconds
            3. 72˚C 30 seconds
        ii. 72˚C 60 seconds (final extension)
        iii. 4˚C hold