PCR*opsis*™ Reagent RVD with RVD Enhancer + PCR*opsis*™ Support enables direct amplification of bacteria from urine

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Abstract:
Urinary tract infections (UTI) are a recurrent problem in nursing homes and other settings. Such infections can lead to serious complications if not properly diagnosed early. Symptomatic UTIs result in at least 7 million visits to outpatient clinics, 1 million visits to emergency departments, and 100,000 hospitalizations annually.[1] People suspected of UTIs undergo PCR testing to determine the presence of abnormal bacteria in their urine. In this report, we show that PCR*opsis*™ Reagent RVD with RVD Enhancer, in combination with PCR*opsis*™ Support, enables extraction-free direct PCR from urine samples containing gram positive or negative bacteria. These reagents were able to process urine samples containing less than 50 cfu / mL of bacteria. PCR*opsis*™ Reagent RVD with RVD Enhancer + PCR*opsis*™ Support offers a robust, low-cost, and fast alternative to nucleic acid extraction for UTI PCR testing.

Results:

URINE	Staphylococcus aureus		Pseudomonas aeruginosa	
	cfu / mL	Ct	**cfu / mL**	Ct
Reagent RVD with RVD Enhancer + Support	**420,000**	20.17	**730,000**	20.52
	42,000	23.05	**73,000**	23.93
	4,200	27.19	**7,300**	26.83
	420	30.89	**730**	29.88
	42	35.22	**73**	33.18
	4.2		**7.3**	38.49

Table 1. Reagent RVD with RVD Enhancer + Support mediates direct PCR amplification of urine samples with less than 50 cfu / mL of S. aureus and P. aeruginosa. The use of these reagents as an alternative to DNA extraction for PCR-mediated detection of diluted bacteria in urine resulted in Ct values ranging from 20 ~ 39. Each sample was tested in duplicates and the indicated Ct is the average of two readings.

UVT	Staphylococcus aureus		Pseudomonas aeruginosa	
	cfu / mL	Ct	**cfu / mL**	Ct
Reagent RVD with RVD Enhancer + Support	**420,000**	20.66	**730,000**	19.83
	42,000	23.55	**73,000**	22.79
	4,200	27.03	**7,300**	25.39
	420	30.44	**730**	29.29
	42	34.19	**73**	34.14
	4.2		**7.3**	36.52

Table 2. Reagent RVD with RVD Enhancer + Support mediates direct PCR amplification of bacterial samples in transport medium with less than 50 cfu / mL of S. aureus and P. aeruginosa. The use of these reagents as an alternative to DNA extraction for PCR-mediated detection of diluted bacteria in transport medium resulted in Ct values ranging from 19 ~ 37. Each sample was tested in duplicates and the indicated Ct is the average of two readings.

Key Conclusions:

- Reagent RVD with RVD Enhancer, in combination with PCR*opsis*™ Support, mediates amplification of gram positive and negative bacteria in urine and transport medium at concentrations less than 50 cfu / mL.

- UTIs usually involve infections with over 10,000 cfu / mL in centrifuged urine.[1,2] The ability to quantitatively detect bacteria in urine over a range expanding more than 400,000 cfu / mL to less than 50 cfu / mL, offers laboratory personal confidence and flexibility in the PCR test.

- The addition of PCR*opsis*™ Support to PCR*opsis*™ Reagent RVD with RVD Enhancer is critical for the amplification of low levels of bacteria in urine. Previous PCR studies with bacteria spiked into urine and PCR*opsis*™ Reagent RVD with RVD Enhancer alone used as an alternative to nucleic acid extraction showed a limit of detection >1000 cfu / mL (data not shown).

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 with RVD Enhancer
    - PCR*opsis*™ Support
    - 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 urine and UVT:

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

Studies with PCR*opsis*™ reagents:

1. Add 2 µL of PCR*opsis*™ Support to 1 mL PCR*opsis*™ Reagent RVD with RVD Enhancer, and mix thoroughly
    a. This mixture is stable for 24 hours

2. Thoroughly mix 20 µL of freshly prepared reagent with 20 µL of urine or UVT samples in a thin walled tube (0.2 mL) and cap tube.
1. Incubate the reagent mixture with bacterial sample for 10 minutes at 95˚C and let cool at room temperature for ~10 seconds before continuing
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:
        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

References:

1. Schappert, SM. Ambulatory care visits to physician offices, hospital outpatient departments, and emergency departments: United States, 1997, *Vital Health Stat 13*, 1999, vol. 143 (pg. i-i) 1–39
2. Rowe, TA & Juthani-Mehta, M. Urinary tract infection in older adults. Aging Health, 2013, 9(5):10.2217.