PCRassays.com

ASSAY NAME: UTI1_BR (Urinary Tract Infection 1 for BioRad)

Quantity: 100 x 20µL PCR reactions

5-plex assay: Enterococcus genus, *Morganella morganii*, *Serratia marcescens*, *Staphylococcus saprophyticus*, and human RPP30 DNA

SKU: BUN-UTI1-D-BR-100 (BioRad)

(RUO). Research Use Only. Not for use in Diagnostic Procedures.

SCOPE OF THIS PIS:

The oligonucleotide recipes are optimized for each instrument (BioRad, QuantStudio). The verification data presented in this PIS were obtained using PNP-UTI1-D-BR-100 on a BioRad CFX96. The performance of the other SKUs on their corresponding instrument should be similar. Contact PCRassays.com if you need to use a different qPCR instrument.

CONTENTS

The primers and probes in the UTI1 assay are provided in Tube 1 as a 5X concentrated working solution that detects 4 pathogens and a human extraction control.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
Enterococcus genus	FAM	BHQ-1	1,2
RPP30-DNA control	HEX	BHQ-1	7
M. morganii	CALFLUOR610	BHQ-2	3,4
S. marcescens	Cy5	BHQ-2	5
S. saprophyticus	Cy5.5	BHQ-2	6

The probes are designed as a TaqMan[8] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity.

ASSAY HANDLING AND CONTAMINATION

The UTI1 bundle is shipped on ice, and should be stored at -20 °C. The tubes should be kept on ice once thawed. Do not subject the enzyme to multiple freeze-thaw cycles.

Any contamination should be avoided by using appropriate personal protective equipment (PPE), powder free gloves, aerosol barrier pipette tips, and a clean hood.

ASSAY CONTENTS:



Tube 1: Primer/Probe mix (5X) for *Enterococcus* genus, *M. morganii*, *S. marcescens*, *S. saprophyticus*, and hRPP30DNA.

Tube 2: (**Do NOT add to specimen unknowns**) Positive control: Synthetic 500 bp DNA fragments of *Enterococcus* genus, *M. morganii*, *S. marcescens*, *S. saprophyticus* and human RPP30DNA.

Tube 3: InhibiTaq qPCR enzyme MasterMix (enough for 100 rxns. with 20 µL total volume). This is a custom formulation from Fortis Life Sciences to the specifications of PCRassays.com.

EXPERIMENTAL

Perform nucleic acid extraction/purification (recommended).

Set up your reaction (20 µL) as follows on ice:

Component	Volume (µL)
InhibiTaq qPCR enzyme mastermix (2X)	10
Primer/Probe mix (5X)	4
Sample	2
Water (Molecular Biology Grade)	4

Notes: To improve assay sensitivity, up to 6 µL of sample can be added (water volume adjusted accordingly) for a total reaction volume of 20 µL. For positive control rxns., add 2 µL of the solution from Tube 2 (i.e., the "sample").
For 10 µL reactions, divide all of the amounts above by a factor of 2.

A PCR protocol was used for verification on a BioRad CFX96 system, with the following program:

Step	Thermocycling Protocol:
1	Incubate @ 95 °C for 2 minutes
2	Incubate @ 95 °C for 3 seconds
3	Incubate @ 55 °C for 15 seconds
4	Plate Read
5	Go to Step 2, repeat 44× more

RESULT INTERPRETATION

After running the qPCR reaction, perform a regression analysis on the data to determine the quantification cycle, Cq. (Cq is preferred over Ct). Each fluorescence channel with a Cq < 38 cycles and final RFU > "threshold" is considered "positive" or "+" in the Table below. The "threshold" is 200 on BioRad instruments and 200,000 on Q.Studio 5, 6, 7, 12K instruments.

Entero. genus FAM™	*Morganella morganii* CALFLUO R610™	*Serratia marcescens* Cy5™	*Staph. saprophyticus* Cy5.5™	RPP30 HEX™	Recommended Interpretation
–	–	–	–	–	The PCR reaction failed. Repeat the experiment.
–	–	–	–	+	The sample contains human RPP30 DNA. The sample doesn't contain bacterial DNA.
+	–	–	–	–	The sample contains *Entero.* genus DNA. The sample may not contain human RPP30 DNA.
+	–	–	–	+	The sample contains *Entero.* genus DNA and human RPP30 DNA.
–	+	–	–	–	The sample contains *M. morganii* DNA. The sample may not contain human RPP30 DNA.
–	+	–	–	+	The sample contains *M. morganii* DNA and human RPP30 DNA.
–	–	+	–	–	The sample contains *S. marcescens* DNA. The sample may not contain human RPP30 DNA.
–	–	+	–	+	The sample contains *S. marcescens* DNA and human RPP30 DNA.
–	–	–	+	–	The sample contains *S. saprophyticus.* DNA. The sample may not contain human RPP30 DNA.
–	–	–	+	+	The sample contains *S. saprophyticus.* DNA and human RPP30 DNA.
+	+	+	+	–	The sample contains *Entero.* genus DNA, *M. morganii* DNA, *S. marcescens* DNA, *S. saprophyticus.* DNA. The sample may not contain human RPP30 DNA.
+	+	+	+	+	The sample contains *Entero.* genus DNA, *M. morganii* DNA, *S. marcescens* DNA, *S. saprophyticus. DNA*, and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The UTI1_BR assay verification was carried out as a 5-plex assay, which simultaneously detects DNA from *Enterococcus* genus, *Morganella morganii*, *Serratia marcescens*, *Staphylococcus saprophyticus.,* and human RPP30 DNA, which serves as a positive control assay.

Experiments were performed in triplicate using the experimental procedure given above, but with different samples added to each reaction. The samples used for the verification experiments contained 1×10^4 copies/reaction of synthetic 500 bp DNA constructs (from Twist Biosciences) harboring the regions of interest from the pathogen genomes, human RPP30 DNA gene, and human genomic DNA. **Figure 1** shows the results of these experiments, which indicate that the 5-plex specifically detects the different pathogens.

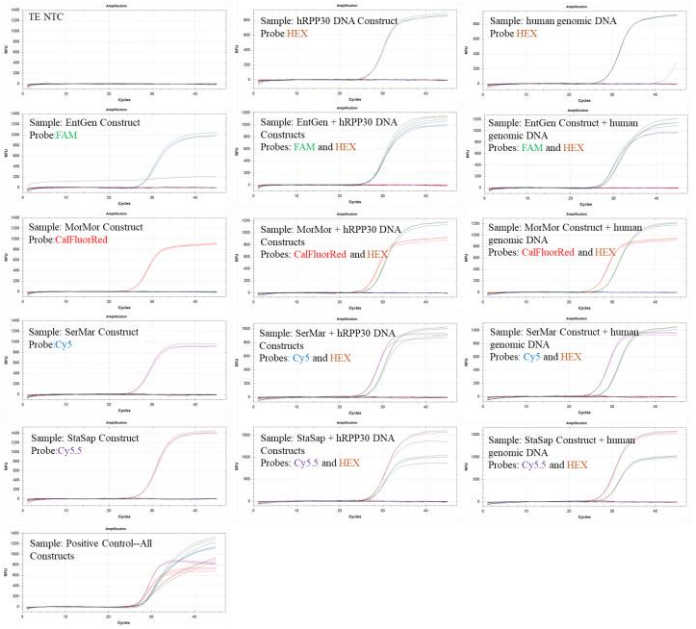


Figure 1: Verification experiments with single and multiple targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

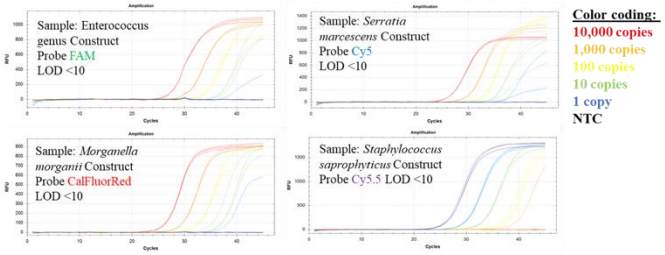


Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

Conclusion: The data in **Figure 1** indicate that the 5-plex primers and probes specifically detect and differentiate the pathogens and are also compatible with RPP30 DNA positive control primers. Human genomic DNA doesn't interfere with the detection of the pathogens.

The limit of detection (LOD) was estimated by performing serial dilution experiments in triplicate (**Figure 2**). For dilution series only target construct was added. The results show a limit of detection (LOD) <10 copies/reaction.

CONTACT US

For assistance, please contact DNA Software using the link: https://www.pcrassays.com/contact/

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NOTES

[1] FAM™ (Carboxyfluorescein), is a trademark of Life Technologies Corporation.

[2] BHQ-1™ (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.

[3] CALFLUOR610™ is a trademark of ThermoFisher Scientific.

[4] BHQ-2™ (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.

[5] Cy5™ is a trademark of GE Healthcare.

[6] Cy5.5™ is a trademark of GE Healthcare.

[7] HEX™ (Hexachlorofluorescein) is a trademark of ThermoFisher Scientific.

[8] TaqMan™ is a trademark of Roche Diagnostics, Inc.