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ASSAY NAME: UTI16_BR
(Urinary Tract Infection panel 16 for BioRad)

Quantity: 100 x 20µL PCR reactions

5-plex assay: *Candida genus, Klebsiella oxytoca, Pseudomonas aeruginosa, Staphylococcus Coagulase Negative,* and human RPP30 DNA

SKU: BUN-UTI16-D-BR-100 (Bio-Rad)

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

SCOPE OF THIS PRODUCT INFORMATION SHEET (PIS):

The oligonucleotide recipes are optimized for each instrument (BioRad, QuantStudio). The verification data presented in this PIS were performed using BUN-UTI16-D-BR-100 on a BioRad CFX96 using white-bottomed plates. The performance of the other SKUs on their corresponding instrument should be similar. Contact PCRassays.com if you need to use a different instrument.

CONTENTS

The primers and probes in the UTI16 assay are provided in Tube 1 as a 5X concentrated working solution that detects 4 pathogens and a human extraction control. Note that the assay for *K. oxytoca* also detects some strains of *K. michiganensis*. The *Candida* genus assay will detect at least 29 species of *Candida* (with alternative names): *C. africana, C. albicans, C. auris, C. bracarensis, C. catenulate, C. dubliniensis, C. duobushaemulonii, C. famata, C. fermentati, C. glabrata (Nakaseomyces glabratus, Cryptococcus glabratus), C. guilliermondii (Meyerozyma guilliermondii, Pichia guillermondii), C. guilliermondii var membranifaciens (Kodamaea ohmeri), C. inconspicua, C. krusei (Pichia kudriavzevii, Issatchenkia orientalis), C. lusitaniae (Clavispora lusitaniae, C. obtusa), C. mesorugosa, C. metapsilosis, C. neorugosa, C. nivariensis, C. norvegensis, C. parapsilosis, C. pararugosa, C. pseudohaemulonii, C. pseudorugosa, C. pulcherrima, C. robusta (Saccharomyces cerevisiae), C. rugosa, C. tropicalis, C. utilis*, and *Torulopsis pintolopesii*. The Staphylococcus Coagulase Negative primers and probe detects the following species: *S. capitis, S. epidermidis, S. haemolyticus, S. hominis, S. lugdungensis, S. pettenkoferi, S. saprophyticus, S. simulans, S. warneri*.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
Candida genus	FAM	BHQ-1	1,2
RPP30-DNA control	HEX	BHQ-1	7
P. aeruginosa	TEX615	BHQ-2	3,4
K. oxytoca	Cy5	BHQ-2	5
Staph. Coag. Negative	Cy5.5	BHQ-2	6

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

Assay contents:

Tube 1: Primer/Probe mix (5X) for *Candida genus, K. oxytoca, P. aeruginosa, Staphylococcus Coagulase Negative*, and hRPP30 DNA.

Tube 2: (**Do NOT add to specimen unknowns**) Positive control: 5000 copies/µl positive controls of synthetic 500 bp DNA fragments of *Candida genus, K. oxytoca, P. aeruginosa, Staphylococcus Coagulase Negative*, and hRPP30 DNA.

Tube 3: InhibiTaq Standard 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.

ASSAY HANDLING

The bacterial UTI16 assay is shipped at ambient temperature, 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.

EXPERIMENTAL

Perform nucleic acid extraction/purification (recommended). Since some of the bacteria in this assay are Gram+, it is important to use an extraction procedure with an appropriate cell wall lysis agent.

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.

A PCR protocol was used for verification on a BioRad CFX96 system using white-bottomed plates, 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). We recommend Cq threshold <37 cycles and a final RFU > 200 (BioRad) is considered "positive" or "+" in the Table below.

P. aerugi nosa TEX 615™	*K. oxytoca* Cy5™	*Staph. Coag. Neg.* Cy5.5™	*Candida genus* FAM ™	hRPP30 HEX™	Recommended Interpretation
–	–	–	–	–	The PCR reaction failed. Please repeat the experiment
–	–	–	–	+	The sample does not contain bacterial DNA of interest. The sample contains human RPP30 DNA.
+	–	–	–	–	The sample contains *P. aeruginosa* DNA. The sample may not contain hRPP30 DNA.
+	–	–	–	+	The sample contains *P. aeruginosa* DNA and human RPP30 DNA.
–	+	–	–	–	The sample contains *K. oxytoca* DNA. The sample may not contain hRPP30 DNA.
–	+	–	–	+	The sample contains *K. oxytoca* DNA and human RPP30 DNA.
–	–	+	–	–	The sample contains *Staph. Coag. Neg.* DNA. The sample may not contain RPP30 DNA.
–	–	+	–	+	The sample contains *Staph. Coag. Neg.* DNA and human RPP30 DNA.
–	–	–	+	–	The sample contains *Candida genus* DNA. The sample may not contain hRPP30 DNA.
–	–	–	+	+	The sample contains *Candida genus* DNA and human RPP30 DNA.
+	+	+	+	–	The sample contains *P. aeruginosa*, *K. oxytoca*, *Staph. Coag. Neg.*, *Candida genus* DNA. The sample may not contain hRPP30 DNA.
+	+	+	+	+	The sample contains *P. aeruginosa*, *K. oxytoca*, *Staph. Coag. Neg.*, *Candida genus* DNA and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The bacterial UTI16 assay verification was carried out as a 5-plex assay that simultaneously detects DNA *Candida genus, K. oxytoca, P. aeruginosa, Staphylococcus Coagulase Negative,* 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. DNA extracts (data not shown) were obtained by ATCC (*C. albicans* #MYA-2876DQ, *C. glabrata* #2001DQ, *C. tropicalis* #750DQ, *S. haemolyticus* #29970D-5, *S. epidermidis* #12228DQ, *S. saprophyticus* #15305DQ). **Figure 1** shows the results of these experiments, which indicate that the 5-plex specifically detects the different 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. For all targets, the results show a limit of detection (LOD) <10 copies/reaction.

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.

NOTES

[1] FAM™ (Carboxyfluorescein), a trademark of Life Tech. Corporation.
[2] BHQ-1™ (Black Hole Quencher) trademark of Biosearch Tech., Inc.
[3] TEX615™ is a trademark of Thermo Fisher Scientific.
[4] BHQ-2™ (Black Hole Quencher) trademark of Biosearch Tech., Inc.
[5] Cy5™, a trademark of GE Healthcare.
[6] Cy5.5™ is a trademark of GE Healthcare.
[7] HEX™ (Hexachloro-fluorescein), a trademark of ThermoFisher Sci.
[8] TaqMan™ is a trademark of Roche Diagnostics, Inc.

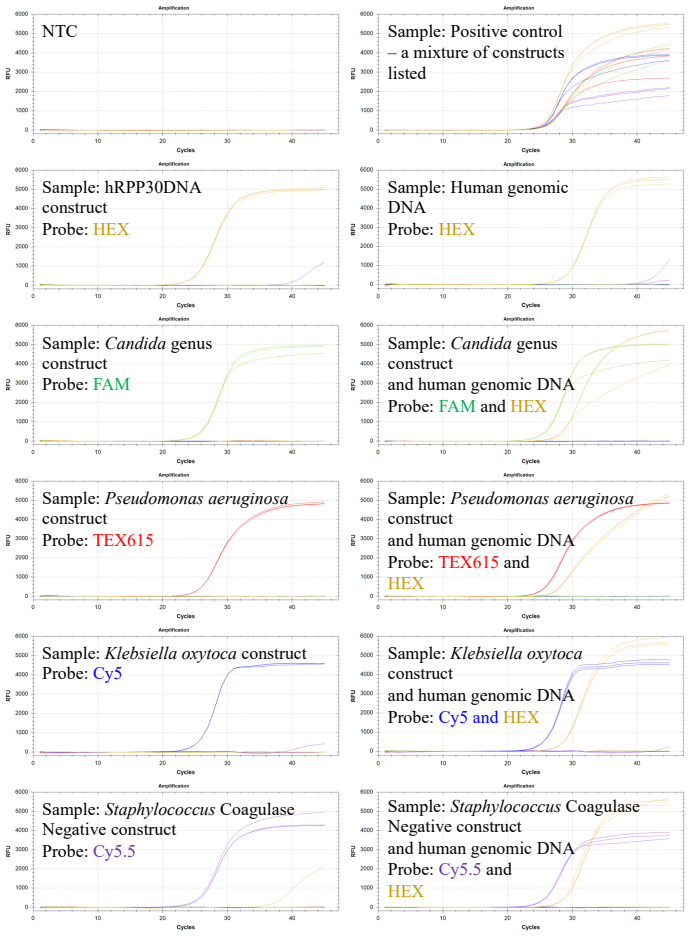

Figure 1: Verification experiments with single targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

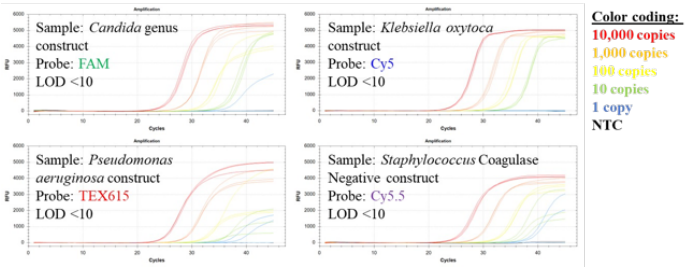

Figure 2: Serial dilution experiments show LOD <10 molecules for all targets.

CONTACT US

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

Address: Michigan Life Science and Innovation Center,
46701 Commerce Center Dr, Plymouth, MI 48170
Phone: (734) 222-9080