Urinary Tract Infection Detection

Culture-based methods for the detection of UTI pathogens lack sensitivity, are subjective, and prolonged Turnaround Times (48-72 Hours). Clinicians frequently treat patients empirically resulting in poor antibiotic stewardship and increased rate of antibiotic resistance. Our new real-time PCR solution for UTI investigations enables rapid detection without the need of culture—while enabling increased sensitivity and specificity of detection of urinary tract pathogens and keeping cost per sample low.
Our real time PCR assays use DNA isolated from specimens such as urine or blood. The product is intended for research use only. Positive results do not rule out bacterial infection or co-infection with other pathogens. Negative results do not preclude microbial infection and should not be used as the sole basis for patient management decisions. Test results must be combined with clinical observations, patient history, and epidemiological information.

SUMMARY AND EXPLANATION OF THE TEST

The *UTI Pathogen Detection Assay* is a real-time PCR test intended for the qualitative detection of DNA from the bacteria and fungus in urine and blood samples.
The assay includes the following urinary tract bacteria targets:
Enterobacter cloacae
Enterococcus faecalis
Escherichia coli
Klebsiella oxytoca
Klebsiella pneumoniae
Morganella morganii
Mycoplasma hominis
Proteus mirabilis
Providencia stuartii
Pseudomonas aeruginosa
Serratia marcescens
Staphylococcus aureus
Staphylococcus saprophyticus
Streptococcus agalactiae
Ureaplasma parvum serovar and Ureaplasma urealyticum
C. albicans; C. glabrata; C. parapsilosis and C. tropicalis
and drug resistance genes:
MecA, MecC, VenA, VenB, KPC, and CTX-M.

PRINCIPLE

The primers and probes are selected from the regions of *targeted conserved regions of the above bacteria*. The primer/probe sets are designed for specific detection of above regions are separated into 4 multiplex qPCR setups. An additional primer/probe set to detect human TERT gene (as an internal control) is also included. DNA extracted from respiratory specimens is amplified in a Real-Time PCR Thermocycler (such as Applied Biosystems 7500 Real-Time PCR Instrument). The fluorescent signal is emitted when the 5' nuclease active domain of Taq polymerase degrades the probe anneals to target sequence between the forward and the reverse primers. During each cycle, the fluorescence intensity is increased with the additional reporter dye molecules released from their respective probes.
The *UTI) Pathogen Detection Assay* also contains a positive control (Plasmid DNA) and a negative control for testing process monitoring.

REAQENTS AND MATERIALS PROVIDED

	Component	Quantity		Volume /T
		100 Tests / Box	500 Tests / Box	
1	UTI PCR Mix I UTI PCR Mix 2 UTI PCR Mix 3 UTI PCR Mix 4 UTI PCR Mix 5 UTI PCR Mix 6 UTI PCR Mix 7	1.6 mL/ tube each	5x1.6mL/tube each	15 µL
2	UTI Positive Control UTI Positive control 6&7	100 µL/ tube × 1	500 µL/ tube × 1	5 µL
3	UTI Negative Control	100 µL/ tube × 1	500 µL/ tube × 1	5 µL

MATERIALS MAY REQUIRED BUT NOT PROVIDED

1. Real-Time PCR Thermocycler compatible with JOE (***VIC or HEX is equivalent to JOE)***, FAM and Cy5 channels.
2. Specimen Collection Containers
3. DNA extraction reagent
4. PCR tubes compatible with Thermocycler.
5. 1.5 mL centrifuge tubes (RNase-Free)
6. Vortex
7. High-speed centrifuge
8. Micropipette (0.5-2 µL, 1-20 µL)
9. Pipette tips with filters
10. Personal protective material

WARNING AND PRECAUTIONS

1. For research use only.
2. Check the expiration date.
3. Follow Good Laboratory Practices: wear appropriate protective clothing and use disposal gloves and protective eyewear. Do not eat, drink, or smoke in designated work areas. Wash hands thoroughly after handling specimens and kit reagents.
4. The testing workflow must be one-directional to minimize contamination risks (allocate segregated areas for each step): it should be started from DNA Purification Area, then move to the Reaction Setup Area, followed by Amplification and Detection Area. Do not bring samples, equipment, and reagents to the area in which the previous step was performed and always change gloves when changing areas.
5. Regular decontamination of commonly used equipment is recommended, especially for micropipettes and work surfaces.
6. Specimens must be treated as potentially infectious sources as well as all reagents and materials that have been exposed to the samples and handled in the same manner as an infectious agent. Take appropriate precautions during specimen collection, storage, handling, and disposal in accordance with the guidance from country or region authorities.
7. To ensure optimal performance of the test, always follow appropriate procedures for specimen collection, transport, storage, and processing. Improper procedures may lead to false negative results.
8. Nucleic acids must first be extracted from specimens using a DNA Extraction Kit prior testing. Do not perform a qPCR Assay directly using specimens without extraction.
9. Appropriate precautions should be exercised to monitor contamination and preserve the purity of kit components and reactions. Avoid microbial and nuclease (RNase/DNase) contamination of specimens and kit components. Avoid the spread of aerosols when handling or uncapping specimens.
10. To minimize cross-contamination, open only one tube at a time in the process.
11. Always change pipette tips between liquid transfers. To minimize cross contamination, it's recommended to use aerosol-barrier pipette tips.
12. Always use disposable gloves and regularly check that they are not contaminated with sample materials. Discard gloves if they become contaminated.

TRANSPORT AND STORAGE INSTRUCTIONS

Store the kit frozen at -20±5°C and avoid repeated freeze-thaw cycles (less than 5 frozen-thaw times allowed). The product is valid for 12 months, and the expiry date of the product is shown on the label.

SPECIMEN TYPE and PRESERVATION

Specimen type: urine or blood samples
Specimen preservation: process specimens for bacterial and fugus NA extraction and nucleic acid detection as soon as possible. Specimens can be stored at 4°C for 24 hours, or frozen at -80°C for longer storage, if not processed immediately. Avoid repeated freeze-thaw cycles during transport and storage of the specimens.

ASSAY PCR REACTION SETUP (at reagent preparation area)

A. Reagent Preparation

Add 15 µL of UIT PCR mix to each PCR tube or well.

B. Addition of Bacteria DNA

Add 5 µL of bacteria DNA extracts of the specimen or positive/negative control to each PCR tube or well according to the reaction preparation table below for testing. Then immediately cap the tube tightly, mix well by vortexing and briefly spin the mixture and move it to the amplification area for PCR detection.
PCR Reaction Preparation:

Component	Volume per test
PCR Mix	15 µL
Negative Control / Positive Control / or DNA of specimen to be tested	5 µL
Total Reaction Volume	20 µL

C. PCR amplification (at Amplification Area, use Applied Biosystems 7500 Real-Time PCR System as an example)

1. Place the complete PCR reaction tubes into the fluorescent quantitative PCR analyzer and label positive control, negative control and testing specimen on the Thermocycler software.
2. Enter the PCR reaction volume: 20 µL.
3. Select fluorescence detection channels: JOE, FAM, Rox and Cy5 channels.
4. Select PCR cycle parameter setting following the instructions below, save the file and start the PCR run.
5. Analyze the results according to your thermocycler manufacturer's instructions. If the positive and negative control samples do not meet their respective requirements, the PCR run must be repeated.

Note: The positive and negative control can be used directly without purification.

Cycles	Step	Time	Temperature
1	Initial denaturation	2 minutes	95 ºC
40	Denaturation	10 seconds	95 ºC
	Annealing/Extension	30 seconds	60 ºC

QUALITY CONTROL

Quality control requirements must be performed in conformance with local, accreditation requirements and the user's laboratory's standard quality control procedures.
NC (negative control) material: no obvious amplification curve for Cy5 and FAM detection channels, and no obvious amplification curve for JOE channel;
PC (positive control) material: obvious amplification curves for Cy5, JOE, ROX detection channels (Ct value ≤37), and obvious amplification curve

for FAM channel;
Pay attention that the above requirements must be met each time when the experiment is performed; otherwise, the experiment is considered as invalidate. The reason of invalidation needs to be resolved and the test result needs to be repeated using residual specimens stored appropriately.

DETERMINATION OF RESULTS

1. The results can only be determined when the results of both controls meet their requirements.
2. Criterion for a positive specimen: a specimen can be reported as positive when FAM, Cy5 and JOE channels are all positive.
3. Criterion for a negative specimen: a specimen can be reported as negative when the JOE channel is positive and the FAM and Cy5 channels are negative.

Multiplex qPCR setup 1

Channel	Bacteria	Results Interpretation
JOE	Serratia marcescens	Serratia marcescens Positive: If JOE has an exponential increase curve with Ct ≤ 37. Klebsiella pneumoniae: If FAM has an exponential increase curves with Ct ≤ 37. Morganella morganii: If ROX has an exponential increase curves with Ct ≤ 37. Mycoplasma hominis: If Cy5 has an exponential increase curves with Ct ≤ 37.
FAM	Klebsiella pneumoniae	
ROX	Morganella morganii	
Cy5	Mycoplasma hominis	

Multiplex qPCR setup 2

Channel	Bacteria	Results Interpretation
JOE	C. glabrata	C. glabrata Positive: If JOE has an exponential increase curve with Ct ≤ 37. Enterobacter cloacae: If FAM has an exponential increase curves with Ct ≤ 37. Staphylococcus aureus: If ROX has an exponential increase curves with Ct ≤ 37. Streptococcus agalactiae: If Cy5 has an exponential increase curves with Ct ≤ 37.
FAM	Enterobacter cloacae	
ROX	Staphylococcus aureus	
Cy5	Streptococcus agalactiae	

Multiplex qPCR setup 3

Channel	Bacteria	Results Interpretation
JOE	Enterococcus faecalis	Enterococcus faecalis Positive: If JOE has an exponential increase curve with Ct ≤ 37. Proteus mirabilis: If Rox has an exponential increase curves with Ct ≤ 37. C. albicans: If Cy5 has an exponential increase curves with Ct ≤ 37.
Rox	Proteus mirabilis	
Cy5	C. albicans	
FAM	Human TERT(optional)	It's a valid result if FAM has an exponential increase curve with Ct ≤37, otherwise it's invalid.

Multiplex qPCR setup 4

Channel	Bacteria	Results Interpretation
JOE	pseudomonas aeruginosa	pseudomonas aeruginosa Positive: If JOE has an exponential increase curve with Ct ≤ 37. Ureaplasma parvum serovar or Ureaplasma urealyticum: If FAM has an exponential increase curves with Ct ≤ 37. Ureaplasma: If Fam has an exponential increase curves with Ct ≤ 37. Escherichia coli: If ROX has an exponential increase curves with Ct ≤ 37. Providencia stuartii: If JOE has an exponential increase curves with Ct ≤ 37.
FAM	Ureaplasma parvum serovar or Ureaplasma urealyticum	
ROX	Escherichia coli	
Cy5	Providencia stuartii	

Multiplex qPCR setup 5

Channel	Bacteria	Results Interpretation
JOE	Staphylococcus saprophyticus	Staphylococcus saprophyticus Positive: If JOE has an exponential increase curve with Ct ≤ 37. C. parapsilosis: If FAM has an exponential increase curves with Ct ≤ 37. Klebsiella oxytoca: If Fam has an exponential increase curves with Ct ≤ 37. C. tropicalis: If ROX has an exponential increase curves with Ct ≤ 37. It's a valid result if Cy5 has an exponential increase curve with Ct ≤37, otherwise it's invalid.
FAM	C. parapsilosis	
ROX	Klebsiella oxytoca	
Cy5	C. tropicalis	

Multiplex qPCR setup 6

Channel	Gene	Results Interpretation
JOE	MecA	MecC Positive: If JOE has an exponential increase curve with Ct ≤ 37. MecA Positive: If FAM has an exponential increase curves with Ct ≤ 37. Ven A and B: If ROX has an exponential increase curves with Ct ≤ 37. KPC positive: If JOE has an exponential increase curves with Ct ≤ 37.
FAM	MecC	
ROX	Ven A and B	
Cy5	KPC	

qPCR setup 7

Channel	Gene	Results Interpretation
FAM	CTX-M	CTX-M Positive: If FAM has an exponential increase curve with Ct ≤ 37.

COMPATIBILITY OF REAL-TIME PCR THERMOCYCLERS

Manufacturer	Model
Applied Biosystems	7500 Real-Time PCR System
	7300 plus Real-Time PCR System
Thermofisher	QuantStudio 5, 6/7 and 12K
BioRad	CFX 96, CFX384

PRODUCT PERFORMANCE

Product analysis performance evaluation results:

1. The analytical sensitivity of this kit is 200 copies/mL.

LIMITATIONS OF TEST

1. This test provides a presumptive assay of UTI infection.
2. Factors that may lead to false negative results must be excluded, including poor specimen quality; specimens collected too early or too late; specimens not properly stored, transported or processed; bacteria or fugus variation, PCR inhibitors, etc.
3. If test results are negative but clinical symptoms persist, follow up with additional serological diagnostic testing.
4. The Assay Procedure and the Interpretation of Test Results sections must be followed closely when testing. Failure to follow the procedure may lead to inaccurate results.
5. This test should be used only with urine and blood specimens. The use of other specimen types has not been validated.
6. The quality of the sample impacts the quality of the test; improper specimen collection, storage and/or transport, and improper DNA purification may yield false negative results.
7. In some samples, extremely low levels of target (below the limit of detection) may yield an amplification signal, but results may not be reproducible.
8. Cross-contamination by samples containing high copies of bacteria and or fugus particles or amplification products from previous reactions can yield false positive results. Take proper precautions to monitor contamination and preserve the purity of the kit components.

Symbols used

Symbol	Meaning
	Manufacturer
EC REP	Authorized representative in the European Community
REF	Catalogue number
LOT	Batch code
Σ	The number of test
	Caution
	Use by date
	Consult instruction for use
	Date of manufacture
	Storage temperature limit

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Version A10

Company name: Biomiga, Inc.
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EC REP

Company name: Lotus NL B.V.
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Intended Use:

Available for **research use only (RUO)**.

Not for use in diagnostic procedures.