REF	**Urinary Tract Infection (UTI) & DR Panel Instruction**	
Σ	**UTI - Urinary Tract Infection PCR Kit**	
IFU	**Information For Use**	
LDT	**Lab Development Test**	
RUO	**For Research Use Only**	
REV #	**DATE:**	**REVISION DESCRIPTION:**
REV:01	**1/28/2023**	**EDITION: V2.0**
REV:02	**8/31/2025**	2ND Release

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Definitions & Abbreviations

Abbreviation	Definition
Ct	Cycle Threshold; the PCR cycle at which fluorescence exceeds baseline.
IC	Internal Control: non-target DNA (Yeast DNA) added to monitor extraction and amplification.
PC	Positive Control; plasmid containing assay target sequences.
NC	Negative Control; reagent blank or plasmid containing IC only.
LoD	Limit of Detection; lowest concentration of target reliably detected.
QC	Quality Control; procedures ensuring run validity.
RUO	Research Use Only; not for use in diagnostic procedures (per 21 CFR 809.10(c)(2)(i)).
GLP	Good Laboratory Practice.
PPE	Personal Protective Equipment.
qPCR	Quantitative Polymerase Chain Reaction (real-time PCR).
UDG	Uracil-DNA Glycosylase; enzyme used to prevent carryover contamination.
dUTP	Deoxy Uridine Triphosphate; used with UDG to eliminate amplicon contamination.
FAM / VIC / ROX / CY5	Fluorescence channels are used for multiplex detection.

Pathogen & Resistances Target Symbols							
	Bacteria		Virus		Fungi		Resistance

⚠ For Research Use Only (RUO). Not for use in diagnostic procedures

List of Contents

⚠ For Research Use Only (RUO). Not for use in diagnostic procedures

1. Product Name

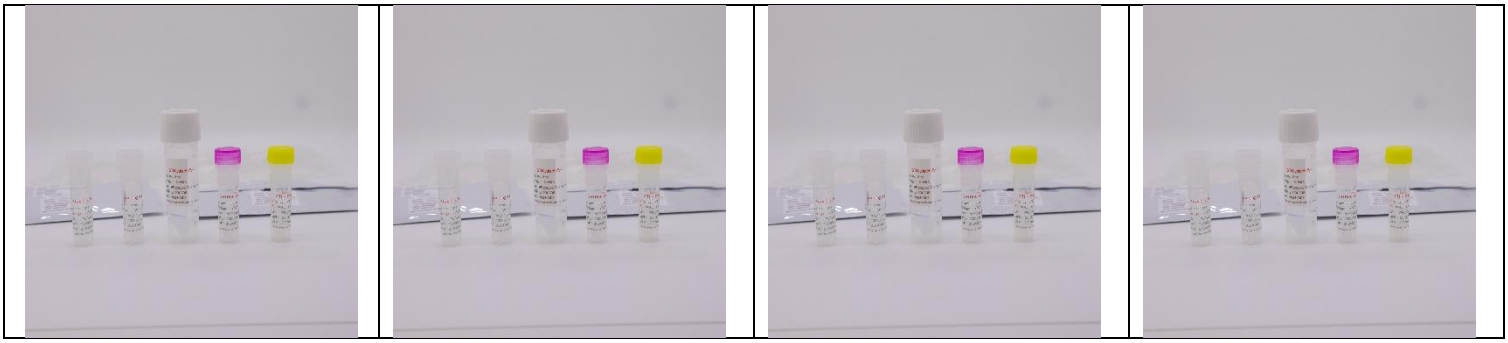
UTI – Urinary Tract Infection Panel (19 bacterial pathogens + resistance genes).

2. Specification

A. Product Name: ReagenX™ UTI-X Multiplex PCR Kit
B. Catalog Number: RX-UTI-001
C. Kit Sizes: 8 tests / 24 tests
D. Format: Lyophilized PCR reagents in 8-well strip tubes

3. Intended Use

A. The ReagenX™ UTI-X Multiplex PCR Kit is intended for the qualitative detection of nucleic acids from bacterial and fungal pathogens commonly associated with urinary tract infections (UTIs), as well as selected antimicrobial resistance genes, using real-time PCR.
B. This product is For Research Use Only (RUO) in accordance with 21 CFR 809.10(c)(2)(i). It is not cleared or approved by the U.S. Food and Drug Administration (FDA) for diagnostic use. Performance characteristics must be established by the end-user laboratory.



4. Principle of the Procedure

A. The ReagenX™ UTI-X Multiplex PCR Kit is designed with specific primers and fluorescent probes for the qualitative detection of common urinary tract infection (UTI) pathogens and clinically relevant antimicrobial resistance genes.
B. Nucleic acids extracted from urine specimens are amplified using real-time polymerase chain reaction (qPCR) with multiplex fluorescent probes, enabling simultaneous detection of pathogen-specific and resistance gene targets.
C. An internal control (IC) derived from edible yeast nucleic acids is included to monitor nucleic acid extraction and amplification efficiency, thereby minimizing the risk of false-negative results.
D. To prevent carryover contamination, the amplification system incorporates a uracil-DNA glycosylase (UDG)/dUTP safeguard, which degrades residual amplicons and reduces the risk of false-positive results.
E. The kit is provided as a fully premixed, freeze-dried system. Each PCR tube contains Taq DNA polymerase, UDG enzyme, reaction buffer, dNTP/dUTP mix, Mg^{2+}, and specific primers/probes.
F. Each strip contains 1–8 lyophilized wells, with each well targeting designated UTI pathogens and/or resistance gene regions.
G. After rehydration with dissolving solution and addition of extracted nucleic acid, amplification is performed directly on a compatible real-time PCR instrument.

5. Pathogen & Resistance Targets

Icon	Category	Target	Example Role in Infection	Notes
🦠	Bacteria	*Escherichia coli*	Major cause of UTIs	Gram-negative
🦠	Bacteria	*Klebsiella aerogenes*	Opportunistic	ESBL potential
🦠	Bacteria	*Klebsiella oxytoca*	Opportunistic	ESBL potential
🦠	Bacteria	*Klebsiella pneumoniae*	Complicated UTIs	ESBL / CRE
🦠	Bacteria	*Citrobacter spp.*	Opportunistic	UTI pathogen
🦠	Bacteria	*Enterobacter cloacae*	Opportunistic	MDR risk
🦠	Bacteria	*Acinetobacter baumannii*	Opportunistic	MDR, biofilm
🦠	Bacteria	*Proteus mirabilis*	Urease-producer	Stone formation
🦠	Bacteria	*Pseudomonas aeruginosa*	Chronic UTIs	Biofilm
🦠	Bacteria	*Serratia marcescens*	Opportunistic	Hospital-acquired
🦠	Bacteria	*Staphylococcus aureus*	Complicated UTIs	MRSA risk
🦠	Bacteria	*Staphylococcus saprophyticus*	Uncomplicated UTIs	Young females
🦠	Bacteria	*Streptococcus agalactiae*	UTI in pregnancy	Neonatal risk
🦠	Bacteria	*Enterococcus faecalis*	Complicated UTIs	VRE risk
🦠	Bacteria	*Enterococcus faecium*	Hospital-acquired	VRE risk
🦠	Bacteria	*Morganella morganii*	Opportunistic	Urease-producer
🦠	Bacteria	*Bacteroides fragilis*	Anaerobe	Complicated infections
🦠	Bacteria	*Staphylococcus epidermidis*	Opportunistic	Device-related
🍄	Fungi	*Candida albicans*	Fungal UTIs	Opportunistic
🧬	Resistance	blaKPC	Carbapenems	CRE
🧬	Resistance	blaNDM	Carbapenems	CRE
🧬	Resistance	blaVIM	Carbapenems	CRE
🧬	Resistance	blaIMP	Carbapenems	CRE
🧬	Resistance	blaOXA-48	Carbapenems	CRE
🧬	Resistance	mecA	Methicillin resistance	MRSA
🧬	Resistance	vanA	Vancomycin resistance	VRE
🧬	Resistance	vanB	Vancomycin resistance	VRE
🧬	Resistance	CTX- M²	ESBL	β-lactam resistance
🧬	Resistance	sul1	Sulfonamide resistance	AMR marker
🧬	Resistance	sul2	Sulfonamide resistance	AMR marker
🧬	Resistance	sul3	Sulfonamide resistance	AMR marker

Table 5. → Pathogen Targets

6. Warnings & Precautions.

A. ⚠ For Research Use Only (RUO). Not for use in diagnostic procedures.

B. Treat all human specimens as potentially infectious. Handle and dispose of them in strict accordance with institutional and regulatory biosafety requirements.

C. Personnel training is mandatory. Laboratory staff must be professionally trained in:

- **Sample collection and specimen handling**
- **Reagent preparation and workflow setup**
- **PCR instrument operation**
- **Data analysis and interpretation**

Laboratory practices must comply with local, national, and international regulations governing molecular diagnostic testing.

D. Laboratory workflow areas should be physically separated to minimize contamination:

- **Reagent preparation area**
- **Sample preparation area**
- **Amplification and analysis area**
- ⚠ **Use** dedicated equipment and consumables **in each area. Cross-use is strictly prohibited.**

E. Personal Protective Equipment (PPE) must be always worn, including:

- **Laboratory coats or gowns**
- **Powder-free disposable gloves**
- **Protective eyewear (goggles or face shield)**
- **Hair covers**
- **Surgical masks or fit-tested N95 respirators**

Ensure full coverage of exposed skin to prevent direct contact with specimens or reagents.

F. Spill or exposure response:

- **Immediately rinse exposed skin or mucous membranes with copious amounts of water.**
- **If reagents contact wounds or eyes, seek medical attention promptly and notify the appropriate health and safety office.**

G. Proper training in PCR workflows and contamination control is essential to ensure accuracy and safety. Always read and fully understand the Instructions for Use (IFU) before performing the Assay.

H. Specimen handling:
All specimen collection and processing must strictly follow applicable regulations and guidelines. Perform all specimen manipulations in a Class II biosafety cabinet to protect operators and prevent environmental contamination.

I. Use only RNase/DNase-free consumables and nuclease-free water during all test procedures to prevent nucleic acid degradation or contamination.

7. Kit Components

Components	8 samples/kit	24 samples/kit	Ingredient
UTI Lyophilized Reagent	8 × 8 strip tubes	24 × 8 strip tubes	Specific primer & probes for the detection of pathogens and DR genes in Table 1, dNTP/dUTP Mix, Mg^{2+}, Taq polymerase and UDG enzyme
UTI Lysis Buffer	1 mL × 1 tube	1 mL × 3 tubes	Surface active agent, balanced salt solution
Internal Control Dry Powder	1 tube	1 tube	Edible yeast powder
Internal Control Solution	1 mL × 1 tube	1 mL × 1 tube	DNase/RNase-free H_2O
One Test Glass Beads	8 tubes	8 tubes × 3 bags	Glass beads
UTI Positive Control	300 µL × 1 tube	300 µL × 1 tube	Plasmid containing every target gene sequence
UTI Negative Control	300 µL × 1 tube	300 µL × 1 tube	Plasmid containing internal control sequence
UTI Dissolving Solution	1 mL × 1 tube	1 mL × 3 tubes	Stablizer

Table 7. → Kit Components

Notes:

1. Do not mix components from different batches.
2. Positive/Negative Controls should be used when contamination or reagent failure is suspected.
3. Treat all **urogenital swabs and urine specimens** as potentially infectious and handle under appropriate biosafety precautions.
4. Perform sample preparation, reagent setup, and amplification in physically separated areas.
5. Use dedicated pipettes and filtered tips; change gloves often, especially after handling specimens.
6. Do not exchange reagents from different lots.
7. Protect fluorescent reagents and PCR tubes from direct light.
8. Dispose of swabs, tubes, and consumables according to biosafety waste regulations (autoclaving, incineration, or chemical disinfection).
9. This kit is RUO; not approved for diagnostic purposes. Use under GLP with trained operators.

7.1 Materials Required but Not Supplied

Equipment / Material	Description	Notes
Class II Biosafety Cabinet	For safe specimen handling and contamination control	Required for molecular diagnostics
Personal Protective Equipment (PPE)	Lab coats/gowns, gloves, protective eyewear (goggles/face shield), surgical or N95 masks	To ensure operator safety
Adjustable Micropipettes	0.5–10 µL, 10–100 µL, 100–1,000 µL ranges	Calibrated, single- or multi-channel
Aerosol-Resistant Filter Tips	RNase/DNase-free	To minimize contamination risk
1.5 mL Microcentrifuge Tubes & Racks	Sterile, RNase/DNase-free	For sample and reagent handling
Benchtop Microcentrifuge (≥12,000 rpm)	For nucleic acid extraction/release	Compatible with 1.5 mL tubes
Vortex Mixer	For specimen and reagent mixing	Power ≥40W recommended
Heating Block / Water Bath (95 °C)	For nucleic acid release	Fits 1.5 mL tubes
Centrifuge Tube Holder (for vortex)	Optional; can replace manual mixing	For consistent bead-based lysis if used
Laboratory Refrigerator (4–10 °C)	For sample and reagent storage	Continuous temperature monitoring recommended
Laboratory Freezer (–20 °C)	For sample/reagent preservation	Avoid repeated freeze–thaw cycles
Collection Swabs & Transport Medium – UTI	Midstream urine specimens collected in sterile transport tubes or UTM/ITM	FDA-cleared/CE-marked. Bioteke Disposable Virus Sampling Swab Kits are recommended.
Real-time PCR Instrument	With FAM, VIC/HEX, ROX, CY5 channels	Compatible systems: ABI 7500, Bio-Rad CFX96, QuantStudio, SLAN-96S, BTK-96

Table 7.1 → Materials Required but Not Supplied

8. Storage & Shelf Life.

A. Transport**:** Store and transport reagents at **room temperature (≤ 1 month)**.
B. Storage**:** Keep kit components at **–20 °C ± 5 °C** for up to **12 months**.
C. Freeze**–thaw:** Avoid more than **7 freeze–thaw cycles**.
D. Light **protection:** Keep lyophilized reagents and fluorescent probes protected from **light exposure**.

9. Instruments.

A. Real-time PCR instruments such as ABI7500, Bio-Rad CFX96, SLAN-96S, QuantStudio and BTK-8

10. Specimen Collection & Handling

10.1 Specimen Types

- First-void urine specimens (male or female).

10.2 Collection

- The patient should be advised not to urinate for at least two hours prior to sample collection. Collect 10-20 mL of clean midstream urine into a sterile container.

10.3 F. Storage

2–8 °C: up to 7-days..
–20 °C: up to 6-months.
–Avoid repeated freeze–thaw cycles.

10.4 Transport

Transport specimens in **sealed insulated containers with ice packs** at 2–8 °C if delivery is within 24–72 hours.
For longer transport times, ship specimens **frozen on dry ice** (–20 °C).

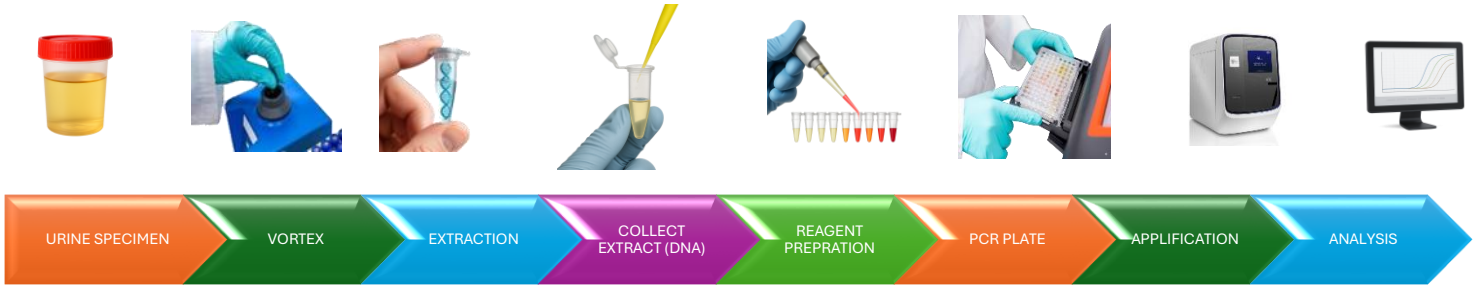
10.5 Rejection Criteria

- Specimens are not collected in VTM/UTM/ITM.
- Leaking, broken, or unlabeled tubes.
- Samples stored beyond allowable stability limits.

11. Test Procedure & Protocol

11.1 Workflow

Urine Specimen (1ml) → Nucleic acid extraction / release → Collect extract (RNA/DNA) → Reagent rehydration (15 μL Dissolving Solution) → PCR setup (add 5 μL extract) → Amplification (qPCR, 40 cycles)
→ Result analysis



According to Corresponding Requirement & Procedures

11.2 Reagent Preparation (Reagent Preparation Area)

A. Take out the **nucleic acid extraction reagent** or **Nucleic Acid Release Reagent** and the components of the kit.
B. Balance them at **room temperature**.
C. Centrifuge liquid items briefly to collect the contents, then set aside for standby use.

11.3 Specimen Processing (Specimen Processing Area)

A. **Nucleic Acid Extraction**

After urine specimens are mixed thoroughly by vortex mixer, take 1mL urine specimen to 1.5mL centrifuge tubes and then add 10μL internal control fluid (Internal Control Dry Powder was mixed with Internal Control Solution)in each tube, and centrifuge at 12000rpm for 10min. After centrifugation, white precipitation can be observed at the bottom of the tube. The supernatant should be carefully discarded and ensure the precipitation remains in the tube.

a. Nucleic acid releasing
   1. Add 100μL UTI Lysis Buffer to the above-mentioned centrifuge tube, and then take One Test Glass Beads and pour them into centrifuge tube.
   2. Cover the tube tightly and oscillate manually for 2 minutes with vortex mixer (power≥40W), or automatically oscillate for 10 minutes on the centrifuge tube holder of vortex mixer.
   3. After oscillation, place centrifuge tube in the metal bath/water bath (preheated in advance) and heat at 95℃ for 2 minutes.
   4. Then centrifuge the tube at 12000rpm for 1min, and nucleic acid are in the supernatant. If the nucleic acid cannot be detected immediately, it can be stored at 2-8℃ for no more than 24 hours, and-20℃ for no more than 1 month

B. **Amplification System Configuration**
- Take out Lyophilized reagent PCR tubes according to the number of samples.
- One strip = detection for 1 sample.
- If Negative and Positive Control tests are required, increase the number of samples by 2.

C. **Stepwise Setup:**
- Add 15 μL Dissolving Solution to each tube to dissolve the lyophilized powder.
- Add 5 μL nucleic acid of the extracted Negative Control / Sample to be tested / Positive Control.
- Total volume of each tube = 20 μL.
- Cap tubes tightly, mix gently by hand (do not vortex).

- Centrifuge briefly at low speed to collect liquid and remove bubbles.

Sample Test Well Preparation	Well 1	Well 2	Well 3	Well 4	Well 5	Well 6	Well 7	Well 8
Dissolving solution	15 µL	15 µL	15 µL	15 µL	15 µL	15 µL	15 µL	15 µL
Extracted nucleic acid of sample	5 µL	5 µL	5 µL	5 µL	5 µL	5 µL	5 µL	5 µL

Table 11.3a → Sample Well Preparation

Negative Control Test Well Preparation	Well 1	Well 2	Well 3	Well 4	Well 5	Well 6	Well 7	Well 8
Dissolving solution	15 µL	15 µL	15 µL	15 µL	15 µL	15 µL	15 µL	15 µL
Extracted nucleic acid of Negative Control	5 µL	5 µL	5 µL	5 µL	5 µL	5 µL	5 µL	5 µL

Table 11.3b: → Negative Control Well Preparation

Positive Control Test Well Preparation	Well 1	Well 2	Well 3	Well 4	Well 5	Well 6	Well 7	Well 8
Dissolving solution	15 µL	15 µL	15 µL	15 µL	15 µL	15 µL	15 µL	15 µL
Extracted nucleic acid of Positive Control	5 µL	5 µL	5 µL	5 µL	5 µL	5 µL	5 µL	5 µL

Table 11.3c → Positive Control Well Preparation

11.4 Run Setup Check List

Item	Requirement	Verified
Reagents equilibrated	All kit components at room temp; probes protected from light	☐
Strip assignment	One 8-well strip per sample; wells 1–8 assigned in order	☐
Reaction volume	15 µL Dissolving Solution + 5 µL nucleic acid = **20 µL** total	☐
Controls included	≥1 Positive Control and 1 Negative Control per run	☐
Pipetting	Always use aerosol-resistant filter tips; avoid vortex after reagent rehydration.	☐
Spin-down	Brief centrifugation to remove bubbles	☐
Instrument channels	**FAM / VIC(HEX) / ROX / CY5** enabled	☐
Program	95 °C 2 min → **40** × (95 °C 10 s; 60 °C 30 s)	☐
Data settings	Baseline Start 3–15; End 5–20; threshold above NC curve	☐

Table 11.4 → Run Setup Check List

11.5 Notes on Instrument Loading

- **ABI7500, CFX96, QuantStudio, SLAN-96S**:
  - The prepared 8-strip tubes can be directly transferred to the amplification detection area.
- **BTK-8**:
  - The mixture from lyophilized reagent PCR strips should be transferred into the chip wells.
  - Hold pipette at 90° vertical, use aerosol barrier tips, pipette into the center, stop at the first stop to avoid bubbles.
  - Seal wells with chip membrane before transferring to amplification area.

11.6 Storage of Remaining Tubes

A. This step is not applicable to UTI Kits**.**
- Each UTI lyophilized strip is fully consumed by a single sample.
- No partial strip storage is required.
- Proceed directly to amplification after setting up.

11.7 PCR Amplification (Detection Area)

A. Put the reaction tubes into PCR instrument and set the names of each reaction well in the corresponding order.
B. Select fluorescence channels and targets corresponding to different tubes according to the following table:

Well	FAM	VIC/HEX	ROX	CY5
1	*Staphylococcus aureus*	*Serratia marcescens*	*Staphylococcus saprophyticus*	*Streptococcus agalactiae*
2	*Enterococcus faecalis*	*Enterococcus faecium*	*Candida albicans*	*Bacteroides fragilis*
3	*Escherichia coli*	*Klebsiella pneumoniae*	*Klebsiella oxytoca*	*Klebsiella aerogenes*
4	*Acinetobacter baumannii*	*Pseudomonas aeruginosa*	*Enterobacter cloacae*	*Proteus mirabilis*
5	*Morganella morganii*	*Staphylococcus epidermidis*	*Citrobacter spp.*	**Internal Control**
6	**blaKPC**	**blaNDM**	**blaVIM**	**blaIMP**
7	**blaOXA-48**	**vanA**	**mecA**	**vanB**
8	**CTX-M**	**sul1**	**sul2**	**sul3**

Table: 11.7 → PCR Amplification (Detection Area)

11.8 PCR Cycling Program

	Steps	Temperature	Time	Cycles
1.	Pre-denaturation	95 ℃	2 min	1
2	Denaturation	95 ℃	10 s	40
	Annealing, extension, fluorescence acquisition	60 ℃	30 s	

Table: 11.8a → Amplification Program of Common Real-Time PCR Instrument

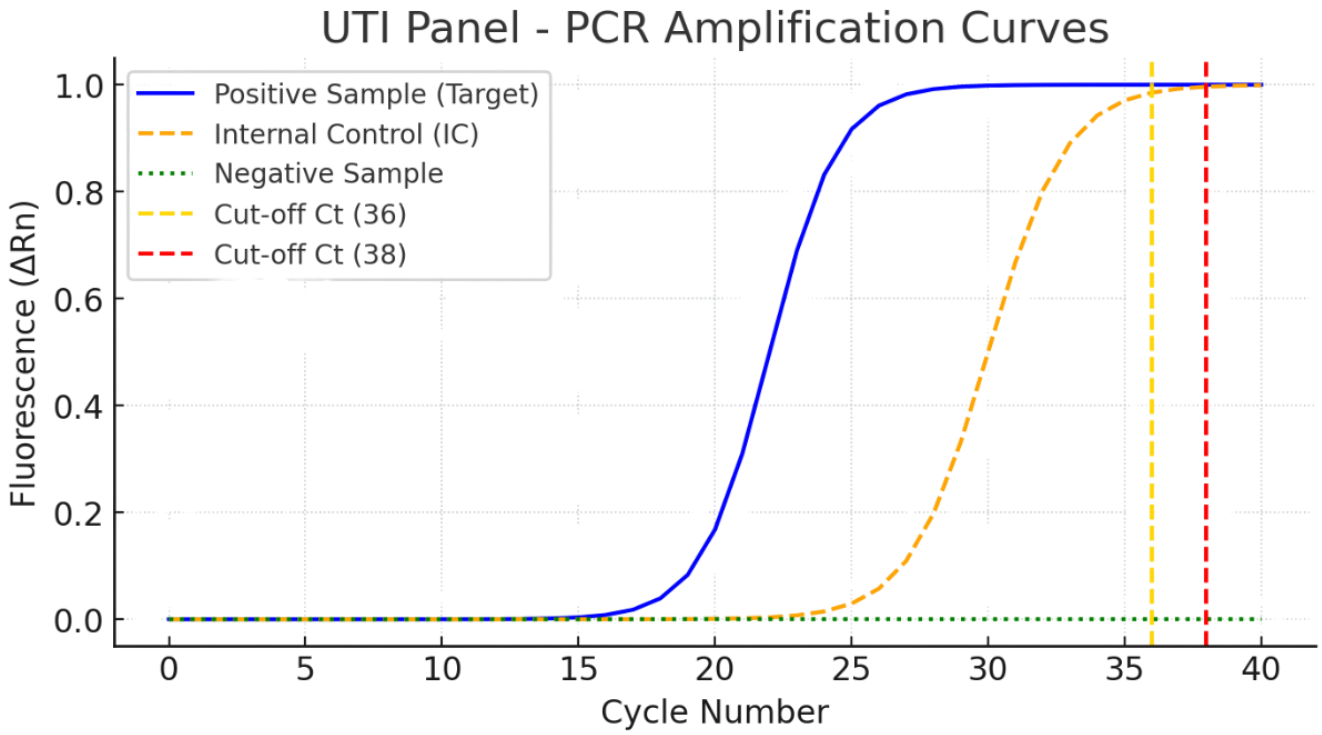
	Steps	Temperature	Time	Cycles
1.	Pre-denaturation	95 ℃	2 min	1
2	Denaturation	95 ℃	10 s	40
	Annealing, extension, fluorescence acquisition	60 ℃	30 s	

Table 11.8b → Amplification Program of BTK-8

11.9 Results Analysis (refer to Instrument User Manual)

After the reaction, the results will be saved automatically.

A. Common real-time PCR instruments:
- Adjust the Start value and End value of the Baseline:
  - Start value: **3–15**
  - End value: **5–20**
- The amplification curve of the Negative Control should be straight or remain below the threshold line.
- Analyze the amplification curves of different detection targets separately, using the corresponding Negative Control.
- Threshold line setting principle:
- Threshold line is recommended to be set just above the highest point of the Negative Control curve and above the fluorescence background value of the sample



B. BTK-8 instruments:
- Analysis is performed using the default program.
- Click “Analysis” to automatically obtain the results.
- Adjust parameters as needed to meet the requirements of Section 5: Quality Control.
- Review the detection results in the Report window.

11.10 Quality Control

The results of negative control and positive control in each tube and channel shall conform to the following table:

Wells	Channel	Negative Control	Positive Control
Wells 1–4	FAM	No Ct or Ct > 38	Ct ≤ 38 with normal amplification curve
	VIC	No Ct or Ct > 38	Ct ≤ 38 with normal amplification curve
	ROX	No Ct or Ct > 38	Ct ≤ 38 with normal amplification curve
	CY5	No Ct or Ct > 38	Ct ≤ 38 with normal amplification curve
Well 5	FAM	No Ct or Ct > 38	Ct ≤ 38 with normal amplification curve
	VIC	No Ct or Ct > 36	Ct ≤ 36 with normal amplification curve (**S. epidermidis**)
	ROX	No Ct or Ct > 38	Ct ≤ 38 with normal amplification curve
	CY5	No Ct or Ct > 38	Ct ≤ 38 with normal amplification curve
Well 6	FAM	No Ct or Ct > 38	Ct ≤ 38 with normal amplification curve (**blaKPC**)
	VIC	No Ct or Ct > 38	Ct ≤ 38 with normal amplification curve (**blaNDM**)
	ROX	No Ct or Ct > 38	Ct ≤ 38 with normal amplification curve (**blaVIM**)
	CY5	No Ct or Ct > 38	Ct ≤ 38 with normal amplification curve (**blaIMP**)
Well 7	FAM	No Ct or Ct > 38	Ct ≤ 38 with normal amplification curve (**blaOXA-48**)
	VIC	No Ct or Ct > 38	Ct ≤ 38 with normal amplification curve (**vanA**)
	ROX	No Ct or Ct > 36	Ct ≤ 36 with normal amplification curve (**mecA**)
	CY5	No Ct or Ct > 38	Ct ≤ 38 with normal amplification curve (**vanB**)
Well 8 (IC)	FAM	No Ct or Ct > 38	Ct ≤ 38 with normal amplification curve (**CTX-M**)
	VIC	No Ct or Ct > 38	Ct ≤ 38 with normal amplification curve (**sul1**)
	ROX	No Ct or Ct > 38	Ct ≤ 38 with normal amplification curve (**sul2**)
	CY5 IC)	**Ct ≤ 38 with normal amplification curve**	**Ct ≤ 38 with normal amplification curve (sul3 + Internal Control)**

Table 11.10 → Quality Control

Notes for Table 11.10 → Quality Control (UTI Panel)

1. **Internal Control (IC, Well 8 CY5)** must amplify with Ct ≤ 38 and a normal curve in all Negative Controls and test samples; otherwise, the sample is invalid.
2. **Negative Control** must show no amplification (No Ct or Ct > 38) in all wells/channels except IC; amplification in other channels indicates contamination and invalidates the run.
3. **Positive Control** must show amplification (Ct ≤ 38 with normal curve) in all assigned channels; failure indicates reagent or instrument issues.
4. Threshold lines should be set just above the highest Negative Control trace (Baseline Start 3–15; End 5–20).
5. Any non-sigmoidal, drifting, or late (>Ct 38) curves should not be interpreted as true positives.
6. If both Positive Control and Negative Control fail, the run is invalid and must be repeated.
7. If multiple pathogen targets amplify in a sample, interpret as mixed infection. Positive results for pathogens can co-exist with positive resistance markers.
8. For *Escherichia coli*, *Staphylococcus epidermidis*, and **mecA**, use a stricter cutoff: Ct ≤ 36 is considered positive.

12. Test Results Interpretation

12.1. Interpretation of Ct value of target gene detection:

Since Well 5 (CY5 channel) contains internal control (IC), its result must be determined before interpreting all other wells.

- If Well 5 yields a valid IC result (Ct ≤ 38 with a normal amplification curve), the test run is considered valid, and the results of Wells (1–4 and 6–8) may then be interpreted.
- If Well 5 does not yield a valid IC result (No Ct or Ct > 38, or abnormal curve), the entire sample test is considered invalid and must be repeated.

Target / Channel	Negative (–)	Positive (+)	Valid with IC	Invalid with IC	Internal Control
Common UTI Pathogen (FAM/VIC/ROX)	No Ct or Ct > 40	Ct ≤ 38 with normal curve	Any results	Any results	—
Drug Resistance Gene (FAM/VIC/ROX)	No Ct or Ct > 40	Ct ≤ 38 with normal curve	Any results	Any results	—
Internal Control (CY5, Well 8)	Ct ≤ 38	—	Required	No Ct or Ct > 38	Required

Table 12.1 → Run Validity & Internal Control Criteria:

Target	Well Position	Channel	Negative (–)	Positive (+)		
				Low	Medium	High
Staphylococcus aureus	Well 1	FAM	No Ct or Ct > 38	30 ≤ Ct ≤ 38	20 < Ct < 30	Ct ≤ 20
Serratia marcescens	Well 1	VIC	No Ct or Ct > 38	30 ≤ Ct ≤ 38	20 < Ct < 30	Ct ≤ 20
Staphylococcus saprophyticus	Well 1	ROX	No Ct or Ct > 38	30 ≤ Ct ≤ 38	20 < Ct < 30	Ct ≤ 20
Streptococcus agalactiae	Well 1	CY5	No Ct or Ct > 38	30 ≤ Ct ≤ 38	20 < Ct < 30	Ct ≤ 20
Enterococcus faecalis	Well 2	FAM	No Ct or Ct > 38	30 ≤ Ct ≤ 38	20 < Ct < 30	Ct ≤ 20
Enterococcus faecium	Well 2	VIC	No Ct or Ct > 38	30 ≤ Ct ≤ 38	20 < Ct < 30	Ct ≤ 20
Candida albicans	Well 2	ROX	No Ct or Ct > 38	30 ≤ Ct ≤ 38	20 < Ct < 30	Ct ≤ 20
Bacteroides fragilis	Well 2	CY5	No Ct or Ct > 38	30 ≤ Ct ≤ 38	20 < Ct < 30	Ct ≤ 20
Escherichia coli	Well 3	FAM	No Ct or Ct > 36	30 ≤ Ct ≤ 36	20 < Ct < 30	Ct ≤ 20
Klebsiella pneumoniae	Well 3	VIC	No Ct or Ct > 38	30 ≤ Ct ≤ 38	20 < Ct < 30	Ct ≤ 20
Klebsiella oxytoca	Well 3	ROX	No Ct or Ct > 38	30 ≤ Ct ≤ 38	20 < Ct < 30	Ct ≤ 20
Klebsiella aerogenes	Well 3	CY5	No Ct or Ct > 38	30 ≤ Ct ≤ 38	20 < Ct < 30	Ct ≤ 20
Acinetobacter baumannii	Well 4	FAM	No Ct or Ct > 38	30 ≤ Ct ≤ 38	20 < Ct < 30	Ct ≤ 20
Pseudomonas aeruginosa	Well 4	VIC	No Ct or Ct > 38	30 ≤ Ct ≤ 38	20 < Ct < 30	Ct ≤ 20
Enterobacter cloacae	Well 4	ROX	No Ct or Ct > 38	30 ≤ Ct ≤ 38	20 < Ct < 30	Ct ≤ 20
Proteus mirabilis	Well 4	CY5	No Ct or Ct > 38	30 ≤ Ct ≤ 38	20 < Ct < 30	Ct ≤ 20
Morganella morganii	Well 5	FAM	No Ct or Ct > 38	30 ≤ Ct ≤ 38	20 < Ct < 30	Ct ≤ 20
Staphylococcus epidermidis	Well 5	VIC	No Ct or Ct > 36	30 ≤ Ct ≤ 36	20 < Ct < 30	Ct ≤ 20
Citrobacter spp.	Well 5	ROX	No Ct or Ct > 38	30 ≤ Ct ≤ 38	20 < Ct < 30	Ct ≤ 20
blaKPC	Well 6	FAM	No Ct or Ct > 38	Ct ≤ 38		
blaNDM	Well 6	VIC	No Ct or Ct > 38	Ct ≤ 38		
blaVIM	Well 6	ROX	No Ct or Ct > 38	Ct ≤ 38		
blaIMP	Well 6	CY5	No Ct or Ct > 38	Ct ≤ 38		
blaOXA-48	Well 7	FAM	No Ct or Ct > 38	Ct ≤ 38		
vanA	Well 7	VIC	No Ct or Ct > 38	Ct ≤ 38		
mecA	Well 7	ROX	No Ct or Ct > 36	Ct ≤ 36		

Table 12.1a → Per-Target Ct Thresholds

Note:

1. If multiple pathogen targets are positive, the results indicate mixed infection with multiple pathogens. While positive for single/multiple pathogens may be combined with positive for single/multiple drug resistance genes.
2. 2. If all the targets are negative and the internal control result meets 6.3, it is interpreted that all targets of the kit are negative.

12.2. Specimen Interpretation Grid – Pathogen-Specific Results

Target Gene / Pathogen	Well 1	Well 2	Well 3	Well 4	Well 5	Well 6	Well 7	Well 8
Staphylococcus aureus	+	±	±	±	±	±	±	±
Serratia marcescens	+	±	±	±	±	±	±	±
Staphylococcus saprophyticus	+	±	±	±	±	±	±	±
Streptococcus agalactiae	+	±	±	±	±	±	±	±
Enterococcus faecalis	±	+	±	±	±	±	±	±
Enterococcus faecium	±	+	±	±	±	±	±	±
Candida albicans	±	+	±	±	±	±	±	±
Bacteroides fragilis	±	+	±	±	±	±	±	±
Escherichia coli	±	±	+	±	±	±	±	±
Klebsiella pneumoniae	±	±	+	±	±	±	±	±
Klebsiella oxytoca	±	±	+	±	±	±	±	±
Klebsiella aerogenes	±	±	+	±	±	±	±	±
Acinetobacter baumannii	±	±	±	+	±	±	±	±
Pseudomonas aeruginosa	±	±	±	+	±	±	±	±
Enterobacter cloacae	±	±	±	+	±	±	±	±
Proteus mirabilis	±	±	±	+	±	±	±	±
Morganella morganii	±	±	±	±	+	±	±	±
Staphylococcus epidermidis	±	±	±	±	+	±	±	±
Citrobacter spp.	±	±	±	±	+	±	±	±
blaKPC	±	±	±	±	±	+	±	±
blaNDM	±	±	±	±	±	+	±	±
blaVIM	±	±	±	±	±	+	±	±
blaIMP	±	±	±	±	±	+	±	±
blaOXA-48	±	±	±	±	±	±	+	±
vanA	±	±	±	±	±	±	+	±
mecA	±	±	±	±	±	±	+	±
vanB	±	±	±	±	±	±	+	±
CTX-M	±	±	±	±	±	±	±	+
sul1	±	±	±	±	±	±	±	+
sul2	±	±	±	±	±	±	±	+
sul3	±	±	±	±	±	±	±	+

Table 12.2 → Specimen Interpretation Grid – Pathogen-Specific Results

13. Assay Limitations

A. The detection result of this kit is only for clinical reference and should not be directly used as the sole evidence for clinical diagnosis or treatment. Clinical management of patients should be considered in combination with their symptoms, medical history, and exposure history.

B. The detection result can be affected by operations including specimen collection, storage, and transportation. False negative results may occur if there are any mistakes in these processes. Cross-contamination during specimen treatment may lead to false positive results.

C. The detected target sequences of this kit are conserved regions of the pathogen genomes. However, sequence variations may lead to false negative results.

D. Pathogens and drug resistance genes not listed in the detection range of this kit cannot be excluded by this method.

14. Performance Characteristics

14.1 Limit of Detection (LoD):

A. ~200 CFU/mL for bacterial pathogens.
B. Resistance genes validated using recombinant plasmids at ~200 copies/reaction.

14.2 Precision (Repeatability & Reproducibility):

A. CV ≤ 5% for Ct values within-run and between-run.
B. Repeatability confirmed across operators and instruments.

14.3 Accuracy (vs. Reference Method):

A. 95–100% agreement with sequencing and culture-based references.
B. Clinical validation demonstrated strong concordance with gold standards.

14.4 Specificity (Cross-reactivity & Interference):

A. Primers/probes in-silico confirmed.
B. Cross-reactions possible: *E. coli* ↔ *Shigella*; *Candida albicans* ↔ *C. dubliniensis*; *S. saprophyticus* ↔ *Serratia spp.*; *K. pneumoniae* ↔ *K. quasipneumoniae/K. variicola*; *K. oxytoca* ↔ *K. michiganensis*.
C. Interference possible from hemoglobin, mucus, antibiotics → recommend local validation.

14.5 Inclusivity (Pathogen Strain Coverage):

A. Covers clinically relevant UTI pathogens (Gram-negative bacilli, Gram-positive cocci, Candida).
B. Resistance genes: blaKPC, blaNDM, blaVIM, blaIMP, blaOXA-48, vanA, vanB, mecA, CTX-M (groups 1/2/9), sul1, sul2, sul3.

15. Attention

A. ⚠ For Research Use Only (RUO). Not for use in diagnostic procedures
B. Transport conditions: The kit must be transported at ≤ 37 °C. If ambient temperature exceeds 37 °C, use insulated containers with ice packs to maintain temperature.
C. Always use sterile, DNase-free and RNase-free consumables (tubes, pipette tips) during testing to prevent contamination.
D. To avoid RNase/DNase contamination, all procedures must be performed in a Class II biosafety cabinet while wearing appropriate personal protective equipment (PPE): disposable gloves, lab coats/gowns, protective eyewear, and surgical or N95 masks.
E. In case of accidental contact with skin or mucous membranes, rinse immediately with plenty of flowing water. If irritation persists, seek medical advice.
F. Before use, ensure all liquid reagents are completely thawed at room temperature, mixed thoroughly, and centrifuged briefly (≥8,000 rpm for a few seconds) to collect contents.
G. After use, all packaging, consumables, and waste liquids must be disposed of as regulated medical waste, following institutional and local biosafety regulations.
H. UTI: Handle all midstream urine specimens as potentially infectious. Follow institutional biosafety guidelines for clinical urine samples.

16. Troubleshooting

Problem	Possible Cause	Solution
Signal in Negative Control	**Contamination during setup**	**Decontaminate workspace, replace consumables, repeat run**
No IC signal (sample negative)	**PCR inhibition or extraction failure**	**Repeat extraction; dilute sample 1:5; verify instrument performance**
High Ct / weak signal	**Low DNA concentration, improper storage**	**Recollect specimen or confirm handling/cold chain**
Positive control fails	**Expired/mishandled kit; PCR program error**	**Use fresh controls; confirm amplification program**
Irregular fluorescence curves	**Bubbles or poor sealing of tubes**	**Centrifuge briefly, reseal caps**
Cross-channel bleed/overlap	**Incorrect baseline/thresholds**	**Reset baselines/thresholds; re-analyze or repeat run**
Many samples invalid	**Workflow contamination or IC not added**	**Review sample prep; confirm IC addition at extraction step**

Table 16. Troubleshooting Guide

17. References

A. Warrell D, Cox TM, Firth JD, Torok E. *Oxford Textbook of Medicine: Infection*. (2012) 6.46.
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C. Yao X, Wang X, Xiuli L, et al. In vitro susceptibilities of aerobic and facultative gram-negative bacilli isolated from patients with intra-abdominal infections and urinary tract infections in China: the 2010 Study for Monitoring Antimicrobial Resistance Trends (SMART). *Proc. 2012 Beijing Laboratory Medicine Annual Meeting and Forum for Young Scientists*.
D. Lipsky BA, Byren I, Hoey CT. Treatment of bacterial prostatitis. *Am Fam Physician*. 2010;50(12):1641–1652.
E. Schwartz DJ, Chen SL, Hultgren SJ, et al. Population dynamics and niche distribution of uropathogenic *Escherichia coli* during acute and chronic urinary tract infection. *Infect Immun*. 2011;79(10):4250–4259.

18. Manufacturer & Distributor

Manufacturer
Bioteke Corporation (Wuxi) Co., Ltd.
Zone A, Floor 4, No. 1719-5, Huishan Avenue,
Huishan Economic Development Zone, Wuxi, Jiangsu, 214174, China
Web: www.bioteke.cn | Email: info@bioteke.cn

Authorized Distributor (U.S.)
Med X Diagnostics LLC.
1800 West Hawthorne Lane – STE P
West Chicago, IL 60185, USA

19. Regulatory Statement

This product is labeled **For Research Use Only (RUO)** in accordance with **21 CFR 809.10(c)(2)(i)**. It is not intended for use in diagnostic procedures. The end-user laboratory is responsible for establishing performance characteristics.

20. Symbols

	Refer to the Manufacturer	**REF**	UTO Panel PCR Kit	**LDT**	Lab Development Test
	Distributor Med X Reagent Solutions	**IVD**	Diagnostic Use	**RUO**	For Research Use Only
IFU	INFORMATION FOR USE	**LOT**	Batch code Use-by date	**EX/REP**	Authorized representative in the European Community
CE	CE mark of conformity				Keep away from Sunlight
	Do not use if package is Temperature limit		Use by date:		Temperature Limit Damaged
COLA	Commission On Laboratory Accreditation	CLIA Certified	Med X Diagnostics is CLIA Certified Diagnostic Lab	CMS	Center of Medicare & Medicaid
	DNA strand (molecular diagnostics		Caution (general hazard warning)	FDA REGISTERED	The Food and Drug Administration Registered
	In vitro diagnostic medical device (⚠ remove if RUO only)		Consult instructions for use		Keep dry
	Date of manufacture For In Vitro		Temperature limit (e.g., –20 °C storage)		Contains sufficient for <n> tests
EU	Authorized representative in the European Community		Keep away from sunlight		Control standard (if supplied)
+ / −	Positive control / Negative control		Keep upright		Do not use if package is damaged
	(Hourglass)		Recyclable packaging		Flammable reagent (if applicable)

⚠ For Research Use Only (RUO). Not for use in diagnostic procedures