LabTurbo AIO Urinary Panel PCR Detection Reagents User Manual

Real-time PCR Detection Reagents

For Research Use Only (RUO)

Catalog Number: Auti08324

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Contents

Intended Use

The LabTurbo AIO Urinary Panel PCR Detection Reagents PCR Detection Reagents are intended for the presumptive qualitative detection of nucleic acid from the Enterobacter aerogenes, Enterobacter cloacae, Enterococcus faecium, Klebsiella oxytoca, Klebsiella pneumoniae, Morganella morganii, Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus saprophyticus, Enterococcus faecalis, Escherichia coli, Streptococcus agalactiae, Acinetobacter baumannii, Citrobacter freundii, Ureaplasma urealyticum, Proteus mirabilis, Proteus vulgaris, Providencia stuartii, Candida albicans, Candida glabrata, Candida tropicalis, Mycoplasma hominis and Staphylococcus aureus in human urine specimens.

Results are for the identification of the DNA of the aforementioned bacteria, which is generally detectable in urine during the acute phase of infection. Positive results are indicative of the presence of DNA of the aforementioned bacteria. Positive results do not rule out the presence of other bacteria or pathogens.

Negative results do not preclude Enterobacter aerogenes, Enterobacter cloacae, Enterococcus faecium, Klebsiella oxytoca, Klebsiella pneumoniae, Morganella morganii, Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus saprophyticus, Enterococcus faecalis, Escherichia coli, Streptococcus agalactiae, Acinetobacter baumannii, Citrobacter freundii, Ureaplasma urealyticum, Proteus mirabilis, Proteus vulgaris, Providencia stuartii, Candida albicans, Candida glabrata, Candida tropicalis, Mycoplasma hominis and Staphylococcus aureus infection and should not be used as the sole basis for presence of the aforementioned bacteria.

The LabTurbo AIO Urinary Panel PCR Detection Reagents are intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

Summary and Pathogen Explanation

Klebsiella aerogenes is a gram negative, rod-shaped bacterium in the Enterobacteriaceae family. Its identification was recently changed from Enterobacter aerogenes due to its close genetic relationship to the Klebsiella family. It is commonly found in the intestines and stool. When Klebsiella spp. infect the urinary tract, symptoms can include fever, chills, pus, swelling, and redness. Transmission of K. aerogenes often occurs in a hospital setting from stool contamination or catheter insertion. Healthy people are typically not at risk for a K. aerogenes infection.

Enterobacter cloacae is a gram negative, rod-shaped bacterium in the Enterobacteriaceae family. It is often found in soil, water, food, and sewage. UTIs caused by Enterobacter spp. typically result in dysuria, frequency and urgency of urination. Both Klebsiella aerogenes and Enterobacter cloacae have been identified as multi-drug resistant pathogens that have been responsible for large hospital-acquired infection outbreaks in recent decades. Because of this, those who stay in hospitals or ICUs for extended periods are most susceptible to an Enterobacter infection.

Enterococcus faecalis is a gram-positive, commensal bacterium commonly found in the gastrointestinal tract, mouth, vagina, and feces. It can cause serious infection when transferred to other areas of the body, including the kidney. Many drug-resistant isolates of E. faecalis have been identified. Among those is VRE, or vancomycin resistant enterococcus. When VREs infect the lower urinary tract, symptoms include dysuria, urinary frequency, and urgency. When VREs infect the upper urinary tract, they can cause fever, vomiting and flank pain.

Enterococcus faecium is a gram-positive bacterium in the Enterococcaceae family. The majority of E. faecium pathogenic isolates have shown antibiotic resistance to vancomycin, ampicillin, and aminoglycosides, increasing the difficulty of treating an infection. Enterococcal infection is the cause for about 15% of UTIs caught in a healthcare setting, and are typically transmitted through contaminated medical devices. Most often, older men suffer from enterococcal infection and those in long-term care or hospital settings are most susceptible.

Klebsiella oxytoca is a gram negative, rod-shaped bacterium in the Enterobacteriaceae family. It is naturally found in the gastrointestinal and respiratory tracts. Catheters are common sources of K. oxytoca infection especially in intensive care units and nursing homes. Transmission of infection typically occurs in healthcare settings, but can also be seen from environmental contamination. Additionally, K. oxytoca has been observed to have multidrug antibiotic resistance.

Among the most common causes for UTI is Klebsiella pneumoniae, a gram-negative, non-motile, rod-shaped bacterium. K. pneumoniae, like other Klebsiella species, is in the Enterobacteriaceae family. It can be found naturally in the intestines and stool. It is spread through direct person-to-person contact and is often spread in hospitals. K. pneumoniae causes typical UTI symptoms including frequency of urination, dysuria, and lower back pain.

Morganella morganii is an increasingly antibiotic-resistant, gram negative, rod-shaped bacterium. Like most other members of the Enterobacteriaceae family, it is found in the natural flora of the gut. Infection of other areas of the body, including the urinary tract, has shown high mortality rates due to multidrug resistance. It is commonly spread in a hospital setting.

Pseudomonas aeruginosa is a gram negative, rod-shaped bacterium in the Pseudomonadaceae family. It can transfer from soil and water to healthcare workers, which in turn can infect patients. It is also commonly associated with biofilm-mediated infection that leads to Catheter Associated Urinary Tract Infection (CAUTI). Therefore, those who stay in hospital settings and those with catheters are most at risk for a P. aeruginosa UTI. Symptoms often include cloudy or bloody urine with an unpleasant smell, painful urination, and pain in the pelvic area.

Serratia marcescens is a gram negative, rod-shaped bacterium in the Enterobacteriaceae family that is often recognized by its red pigment. 30-35% of patients with a S. marcescens UTI are asymptomatic. However, overall mortality rates for infection are high, ranging from 25-58%. Its ability to produce a β-lactamase leads to resistance of β-lactam antibiotics, therefore complicating treatment of infection. It is naturally found in water but has caused numerous outbreaks in intensive care units.

Staphylococcus saprophyticus is a gram-positive bacterium that causes the most common bacterial infection in women. In addition to the gastrointestinal tract, it can be naturally found on the perineum, rectum, cervix, vagina, and urethra. S. saprophyticus infection of the urinary tract typically occurs in people with vaginas shortly after sexual intercourse or menstruation. The majority of cases will present with symptoms like dysuria, frequency or urgency. Other common symptoms are blood and pus in the urine.

80-90% of all UTIs are caused by Escherichia coli, a very common gram-negative organism in the Enterobacteriaceae family. E. coli often passes from the intestines and stool, where it is naturally found, to the urethra and bladder of women due to improper wiping after using the bathroom, sexual intercourse, or pregnancy. Female anatomy allows for easy transfer of fecal bacteria to the urinary tract. A recent concern has been that of ESBL E. coli, or extended-spectrum β-lactamase E.

coli, which has resistance to many antibiotics that typically cure UTIs. ESBL E.coli are more likely to infect those with recurring UTIs, catheters, and those who have recently taken a round of antibiotics.

Streptococcus agalactiae is a gram-positive bacterium in the Streptococcaceae family. Also known as GBS (Group B Streptococcus), this organism is often associated with severe infection in newborns and the elderly. However, GBS can also cause UTIs in young women with a sexually transmitted disease or diabetes. Besides for these susceptible groups, S. agalactiae is typically harmless to healthy adults.

Acinetobacter baumannii is a gram-negative and non-motile bacterium in the Moraxellaceae family. Those who have had long hospital stays or are immunocompromised are most susceptible to A. baumannii infection due to its nosocomial and opportunistic qualities. Symptoms related to an acinetobacter UTI include painful urination, bloody, or cloudy urine, and altered mental status. Multidrug resistance is a recent concern of this organism and has caused increased mortality for infected patients.

Citrobacter freundii is among the most common urinary pathogens. It is a gram-negative bacterium in the Enterobacteriaceae family. It is commonly associated with healthcare settings, and is especially dangerous in pediatric, immunocompromised, and elderly male patients. Any genitourinary instrumentation, including catheterization, increases the chances for a C. freundii infection. Other dangers of C. freundii are meningitis and intestinal infection. Antibiotic resistance is also of concern. Most isolates of C. freundii are resistant to the common UTI antibiotics, including penicillins, cephalosporins, aminoglycosides, and fluoroquinolones.

Ureaplasma urealyticum is a very small bacterium in the Mycoplasmataceae family that stains gram negative because of its lack of a cell wall. It is commonly found in the urogenital tract and is often commensal in the vagina. When populations of U. ureaplasma grow too large, it can cause infection in these regions. It is a difficult organism to culture, so diagnosis via PCR testing is much more accurate. It may be the cause of urinary stone development and tends to be difficult to treat by antibiotics due to its lack of a cell wall.

Proteus mirabilis is a Gram-negative, facultatively anaerobic, bacterium in the Enterobacteriacee family. Patients with long-term catheters are most at risk for a UTI of P. mirabilis. Not only can infection present asymptomatically in elderly patients or those with type 2 diabetes, but it can progress to be life-threatening and cause urosepsis. It can also cause urinary stones and become encapsulated in the stones, therefore protecting it from antibiotic treatment. Like many other pathogens, P. mirabilis has shown

resistance to several common antibiotics, including β-lactams, fluoroquinolones, nitrofurantoin, aminoglycosides, and more.

Proteus vulgaris is a gram-negative bacterium in the Enterobacteriaceae family. It is naturally found in the intestinal tract, soil, water, and feces. Infection of Proteus vulgaris is often seen in those with indwelling catheters and urinary tract abnormalities. A characteristic symptom of a proteus UTI is the formation of struvite stones. P. vulgaris is chromosomally resistant to many aminopenicillins and cephalosporins, but can often be treated with other antibiotics like fluoroquinolones.

Providencia stuartii is a gram-negative bacillus in the Morganellaceae family. It is often found in soil, water, and sewage. P. stuartii has been observed as an opportunistic pathogen primarily affecting those in nursing homes, long-term hospitalized patients, and long-term catheter users. This bacterium tends to adhere to catheter surfaces and cause buildup, blockages, and urinary stones. High mortality rates have been seen in patients with providencia bacteremia due to β-lactam resistance.

Candida albicans is an opportunistic and pathogenic yeast in the Saccharomycetaceae family. It is found in small quantities on the skin, mouth, and intestines, but can cause infection when it grows out of balance with the natural flora. Particularly in women, C. albicans can be found colonizing on the urethral opening. Immune deficiencies can allow C. albicans to grow out of balance, causing urinary tract infection. About 7% of C. albicans tested at the CDC have been identified as resistant to fluconazole, an antifungal drug commonly used to treat infection. Infection is most commonly seen in healthcare settings, and those with female urogenital anatomy and the immunodeficient are more likely to suffer from a C. albicans UTI.

Candida glabrata is a small yeast, measuring at around 2 to 3 microns in diameter. Like all Candida species, it is a fungus in the Saccharomycetaceae family. The GI tract, mouth, and genitals commonly have some C. glabrata, even in healthy people. It is often the cause of UTI in elderly hospitalized patients or those with catheters who have undergone an antimicrobial treatment. Those with suppressed immune systems are also at risk of overgrowth and infection of the yeast. C. glabrata is highly resistant to fluconazole, and recent studies show increasing resistance to Echinocandin, another antifungal drug.

Candida tropicalis is often found on the skin, digestive tract, and female genitourinary tract. It is often transmitted from healthcare workers in hospitals to their patients. People with immunodeficiencies are most at risk for complications associated with candidiasis, or infection from a candida species. C. tropicalis is a common cause of yeast infection in the vaginal cavity. Additionally,

it is a strong biofilm producer, allowing it to adhere to epithelial cells and cause infection. A UTI caused by C. tropicalis can typically be treated with an antifungal drug.

Mycoplasma hominis, along with ureaplasmas, is one of the smallest living organisms we know of. It is in the Mycoplasmataceae family and has the ability to penetrate human cells. Due to its lack of a cell wall, it stains gram negative and is resistant to β-lactams and vancomycin. M. hominis infection is often identified in the genitourinary tract. Infection often occurs during childbirth or abortion. If infection is transferred to a newborn, they can develop meningitis and brain abscesses. Urethral catheterization can also lead to UTI of M. hominis. Infection can typically be cleared with the drug Doxycycline.

Staphylococcus aureus is a Gram-positive round-shaped bacterium in the Staphylococcaceae family. It is often found on the skin and within the respiratory tract. Patients in long-term care facilities, intensive care units, or nursing homes are more susceptible to infection. Indwelling catheters and other invasive urinary tract procedures can lead to S. aureus UTI. Common symptoms include fever and altered mental state. MRSA, or methicillin-resistant Staphylococcus aureus, is a recent clinical concern due to its resistance to β-lactams.

Warnings and Limitations

- LabTurbo AIO Urinary Panel PCR Detection Reagents are for research use only.
- This test has not been FDA cleared.
- This test is only for the detection of nucleic acid from Enterobacter aerogenes, Enterobacter cloacae, Enterococcus faecium, Klebsiella oxytoca, Klebsiella pneumoniae, Morganella morganii, Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus saprophyticus, Enterococcus faecalis, Escherichia coli, Streptococcus agalactiae, Acinetobacter baumannii, Citrobacter freundii, Ureaplasma urealyticum, Proteus mirabilis, Proteus vulgaris, Providencia stuartii, Candida albicans, Candida glabrata, Candida tropicalis, Mycoplasma hominis and Staphylococcus aureus, not for any other viruses or pathogens.
- Negative results do not preclude infection with the aforementioned bacteria and should not be used as the sole basis for bacterial identification.
- Positive detections are indicative of the presence of DNA of the aforementioned bacteria.
- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
- All samples shall be considered potentially infectious and shall be operated and handled in strict accordance with the laboratory's bio-safety requirements. The laboratory personnel should receive professional training (including sample processing, reagent preparation, instrument operation, and software setting, etc.). For the laboratory management specifications, please strictly follow the relevant management specifications for gene amplification test laboratories issued by local regulatory agencies.
- The laboratory should have dedicated reagent preparation area, sample preparation area, and PCR area. Each area should be used solely for its own purpose to avoid contamination.
- In all test procedure, well laboratory and system operation training for laboratory personnel are essential to guarantee the test accuracy and safety. Please read the manual carefully before the experiment.
- Specimen sampling and processing should be performed according to the recommendation of local regulation. Sample processing shall be carried out in the biosafety cabinet to protect the safety of operators and prevent environmental contamination.

- RNase / DNase free pipette tips and water are recommended in all test procedure.
- Avoid swallowing or contacting skin and eyes with Proteinase K/Lysis/Wash Buffer. In case of accidental swallowing or contact, please rinse with tap water and seek medical care.

Principle and Methods

The LabTurbo AIO Urinary Panel PCR Detection Reagents are for multiplex real-time polymerase chain reaction (qPCR) test. The primer and probe set is designed to detect DNA from the Enterobacter aerogenes, Enterobacter cloacae, Enterococcus faecium, Klebsiella oxytoca, Klebsiella pneumoniae, Morganella morganii, Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus saprophyticus, Enterococcus faecalis, Escherichia coli, Streptococcus agalactiae, Acinetobacter baumannii, Citrobacter freundii, Ureaplasma urealyticum, Proteus mirabilis, Proteus vulgaris, Providencia stuartii, Candida albicans, Candida glabrata, Candida tropicalis, Mycoplasma hominis and Staphylococcus aureus in urine specimens from human urine specimens. The Bacillus atrophaeus is used as an extraction control to validate successful extraction of Gram-positive bacteria bacteria from urine; RNase P (RP) gene is used as an internal control to validate successful qPCR reaction in each well.

The assays were established by the full automation method using LabTurbo AIO SP-qPCR System (LabTurbo AIO), LabTurbo stool DNA Mini kit (LSD480-400) for nucleic acid extraction and LabTurbo AIO Urinary Panel PCR Detection Reagents for qPCR assay.

Material and Equipment

Equipment

Nucleic Acid Extraction	PCR Reaction Setup Liquid-Handling	Real-time PCR
LabTurbo SP-qPCR All-in-one system (LabTurbo AIO 48)		

Kits

Kits	
Nucleic acid extraction	LabTurbo stool DNA mini kit (LSD480-400)
PCR kit	LabTurbo AIO Urinary Panel PCR Detection Reagents (Auti08324)
PCR reaction	8-strip PCR white tubes and clear caps

Kit Content

LabTurbo UTI DNA Testing Kit (Auti24)		
Component	Description	Quantity
Primer/Probe mixture UTI 1, 2, 3, 4, 5, 6, 7, and 8	For recognization of the specific bacterial nucleic acid targets	1 X 120 µl (20 X) for each kind of multiplex master mix. 8 kinds of multiplex master mixes are included
PCR Master Mix (MM)	For polymerase chain reaction	6 X 1.6 ml (2 X)
Dilution buffer (DB)	For diluting the master mix components and master mix preparation. Including the primer/ probe mixture for internal control RNaseP gene	6 x 640 ul (1X)
Positive Control (PC)	DNA mixture of all the targets	1 x 12 µl (50 X)
RNase-free water	For PCR negative control	1 x 1.6 ml

Detection Target and Fluorophore

Well No.	Fluorophore	Pathogen Type	Measurand
1	HEX	Gram-positive bacteria	Enterococcus faecium
	FAM	Gram-negative bacteria	Enterobacter aerogenes
	TEXAS RED	Gram-negative bacteria	Enterobacter cloacae
	Cy5.5	Internal control	RNaseP
2	HEX	Gram-negative bacteria	Morganella morganii
	FAM	Gram-negative bacteria	Klebsiella oxytoca
	TEXAS RED	Gram-negative bacteria	Klebsiella pneumoniae
	Cy5.5	Internal control	RNaseP
3	HEX	Gram-positive bacteria	Staphylococcus saprophyticus
	FAM	Gram-negative bacteria	Pseudomonas aeruginosa
	TEXAS RED	Gram-negative bacteria	Serratia marcescens
	Cy5.5	Internal control	RNaseP
4	HEX	Gram-positive bacteria	Streptococcus agalactiae
	FAM	Gram-negative bacteria	Escherichia coli
	TEXAS RED	Gram-positive bacteria	Enterococcus faecalis
	Cy5.5	Internal control	RNaseP
5	HEX	Gram-negative bacteria	Ureaplasma urealyticum
	FAM	Gram-negative bacteria	Acinetobacter baumannii
	TEXAS RED	Gram-negative bacteria	Citrobacter freundii
	Cy5.5	Internal control	RNaseP
6	HEX	Gram-negative bacteria	Providencia stuartii
	FAM	Gram-negative bacteria	Proteus mirabilis
	TEXAS RED	Gram-negative bacteria	Proteus vulgaris
	Cy5.5	Internal control	RNaseP
7	HEX	Fungus	Candida tropicalis
	FAM	Fungus	Candida albicans
	TEXAS RED	Fungus	Candida glabrata
	Cy5.5	Internal control	RNaseP
8	HEX	Gram-negative bacteria	Mycoplasma hominis
	FAM	Gram-positive bacteria	Staphylococcus aureus
	TEXAS RED	Gram-positive bacteria	Bacillus atrophaeus
	Cy5.5	Internal control	RNaseP

Controls Used for LabTurbo AIO UTI Testing Method

1. A "no template" negative extraction control (NEC) is needed to validate reactions from cross contamination and non-specific signal during extraction and qPCR and is used in each batch of qPCR reaction.

2. An internal control (IC) RNase P (RP) gene is needed to validate the extraction procedure and is used to validate the performance of qPCR reaction.

3. An extraction control (EC) Bacillus atrophaeus is a gram-positive bacteria needed to validate the extraction procedure.

4. (Optional) A "no template" (negative) control (NC) is needed to validate reactions from cross contamination and non-specific signal in qPCR procedure and is used for troubleshooting when NEC is invalid in testing. The NC is optional when NEC is used.

5. A positive template control (PC) is needed to validate the correctness of reagent in qPCR procedure and is used at 1000 copies/ul for qPCR reaction.

Reagent Preparation Guide

Extraction reagent preparation

For the use of LabTurbo LSD480-400 kit on LabTurbo AIO SP-qPCR 48 system, follow the step below for extraction reagent preparation.

Buffer LW1: add 230 ml of absolute ethanol to 175 ml of concentrated LW1 buffer bottle. Mix by inverting 10 times.

All the other reagents provided in the kit are ready to use.

Master Mix Preparation

Follow the table below for preparing the 8 master mixes. All 8 master mixes uvse the same formula below.

Component	Multiplex UTI Pathogen Detection	Volume for n Samples
2X PCR Master Mix	10.0 µl	(n+2) x 10.0 ul
Primer/probe mixture	1.0 µl	(n+2) x 1.0 ul
Dilution buffer	4.0 ul	(n+2) x 4.0 ul
Total Target Tube I Mixture	15.0 µl	(n+2) x 15.0 ul
Extract volume added to each reaction	5.0 ul	
Total volume for each reaction	20 ul	

Prepare 2 additional reactions for system operating dead volume. Place the prepared master mixes in 1.5 ml screw cap tubes for the use on LabTurbo AIO 48 system.

Workflow for Running LabTurbo Urinary Panel Using LabTurbo SP-qPCR AIO 48 System

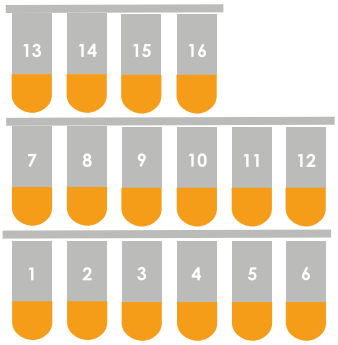
1. On the system monitor, select the sample number and the Urinary panel procedure.

2. Place filtered tips, 6-trip sample tubes, 6-strip column sets, 6-strip elution tubes, 8-strip PCR caps and tubes on the LabTurbo AIO 48 worktable following the instruction on the monitor.

3. Place extraction reagents RLL, CCEB, EtOH and reconstituted LW1 on the LabTurbo AIO 48 system door, and place the Proteinase K vial on the PK rack on the worktable.

4. Place 8 master mix vials and the PC vial on LabTurbo 9-well rack matching the following layout:

5. Place the urine specimen on the worktable and key in the specimen barcode. Place the samples on the system thermal block following the layout below:



6. Click "Start" to initiate the urinary panel pathogen nucleic acid extraction and PCR detection.

Extraction Setting

Urine specimen input volume: 400 ul

Elution volume: 160 ul

PCR Setup Liquid-handling Setting

Master mix volume: 15 ul

Extracted DNA volume: 5 ul

PCR Thermal Profile Setting

	Activation	Denature	Anneal & Elongation
Temperature (°C)	95	95	60
Time (sec)	60	10	30
Cycle (s)	1	40	

PCR Well Layout on LabTurbo AIO Software for 1 Sample

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
A	1	NEC	PC	1	NEC	PC	1	NEC								
B	PC	1	NEC	PC	1	NEC	PC	1								
C	NEC	PC	1	NEC	PC	1	NEC	PC								
D																
E																
F																
G																
H																
I																

PCR Well Layout on LabTurbo AIO Software for 16 Samples

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
A	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
B	9	10	11	12	13	14	15	16	9	10	11	12	13	14	15	16
C	NEC	PC	1	2	3	4	5	6	NEC	PC	1	2	3	4	5	6
D	7	8	9	10	11	12	13	14	7	8	9	10	11	12	13	14
E	15	16	NEC	PC	1	2	3	4	15	16	NEC	PC	1	2	3	4
F	5	6	7	8	9	10	11	12	5	6	7	8	9	10	11	12
G	13	14	15	16	NEC	PC	1	2	13	14	15	16	NEC	PC	1	2
H	3	4	5	6	7	8	9	10	3	4	5	6	7	8	9	10
I	11	12	13	14	15	16	NEC	PC	11	12	13	14	15	16	NEC	PC

Results Interpretation

The NEC control is specifically designed for validating the whole procedure of the assay. If the NEC is invalid, the provided PC and NC can be used for further trouble-shooting in order to inspect all of the possible compromises that could happen in the assay.

1. Negative Extraction Control (NEC): The NEC is the RNase-free water used in molecular biology applications. One reaction is needed for each batch of the assay. All amplification curves in NEC reactions should be negative or with Ct value higher than 35 except the internal control signal. The internal control signal should have positive Cy5.5 detection. If NEC is invalid, it might be due to cross-contamination during handling, improper setup of qPCR assay or degradation of probe. The batch of assay is considered invalid and the whole batch should be repeated from extraction.

2. Internal Control (IC): The recombinant RNase P (RP) DNA spiked into each sample serves as an internal control for each specimen. The Primer/Probe mixture reagent should react with the RP spiked into the urine specimen to generate a positive Cy5.5 detection for each well (Ct value should be below 35). If IC is invalid, re-extract the specimen.

3. Extraction Control (EC): The Bacillus atrophaeus is a gram-positive bacteria and is spiked into each sample serves as an extraction control for each specimen. The extraction processing should successfully digest the gram-positive bacterial cell wall and purify the bacterial DNA from urine specimen. The Primer/Probe mixture reagent should react with the Bacillus atrophaeus spiked into the urine specimen to generate a positive Texas Red detection for well #8 (Ct value should be below 35). If EC is invalid, re-extract the specimen.

4. Negative Control (NC): The NC is the RNase-free water used in molecular biology applications. NC is needed only when NEC is invalid in a batch of testing. All amplification curves in NC reactions should be negative or with Ct values higher than 35. If NC is invalid, it might be due to cross-contamination during handling, reagent preparation, improper setup of qPCR assay or degradation of probe. The batch of assay is considered invalid and the whole batch should be repeated from PCR.

5. Positive Control (PC): The PC is the positive control provided in the LabTurbo AIO UTI testing kit. It is needed for each batch of the assay. The Primer/Probe mixture reagent should react with the PC and generate a positive detection for each target (Ct values should be below 35). If the PC generates a positive result, the reagents and RT-qPCR setup are correct.

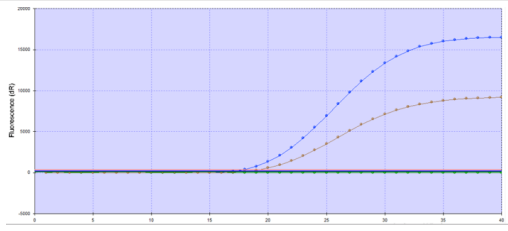
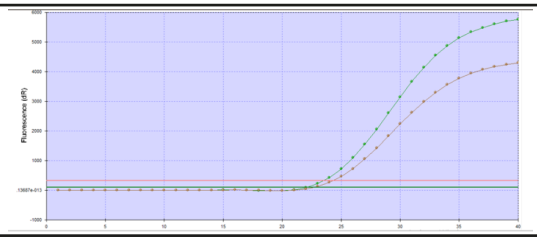
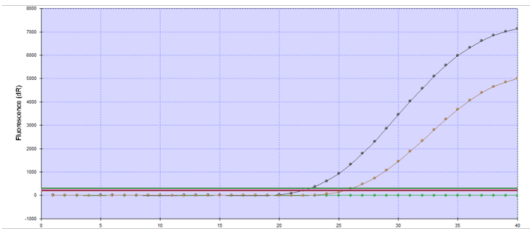
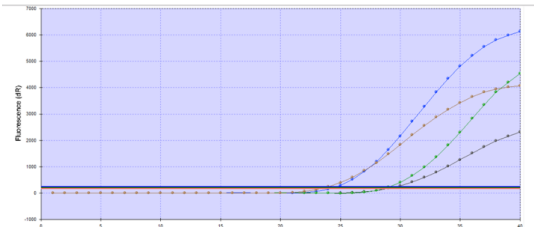
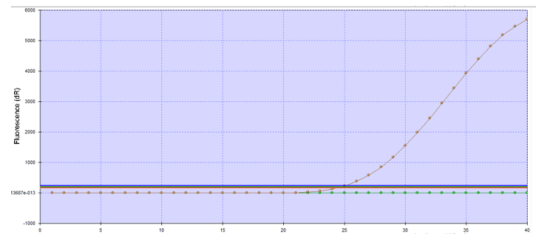
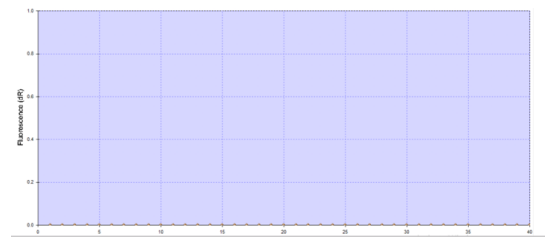
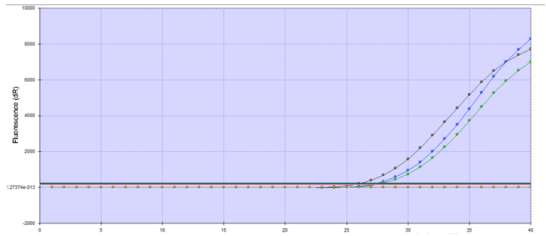
Expected Control Results

Control Type	Control Target	Used to Monitor	Expected Ct Values
Negative Extraction Control (NEC)	-	Non-specific signal and Cross-contamination during DNA extraction, qPCR reaction setup and RT-qPCR	Not detected Or >35.00 Ct for all pathogenic targets. Detected for internal control target.
Internal Control (IC)	RNaseP gene	Correct extraction and qPCR efficiency	< 35.00 Ct
Extraction Control (EC)	Bacillus atrophaeus	Correct bacteria extraction	< 35.00 Ct
Negative Control (NC)	-	Non-specific signal and Cross-contamination in qPCR reaction setup	Not detected Or > 35.00 Ct
Positive Control (PC)	Each pathogen target of LabTurbo AIO UTI testing method	Correct PCR reagent, PCR setup, and qPCR performance	< 35.00 Ct

Expected Sample Results

Pathogen Targets (FAM, HEX, Texas Red)	Bacillus Atrophaeus (Texas Red)	Internal Control RNaseP (Cy5.5)	Negative Extraction Control and Negative Control (NEC & NC)	Result	Interpretation and Action
+ (Ct < 35)	+ (Ct < 35)	+ (Ct < 35)	- (Ct > 35)	Valid	One or multiple UTI pathogen(s) detected
- (Ct > 35)	+ (Ct > 35)	+ (Ct < 35)	- (Ct > 35)	Valid	UTI pathogen not detected
- (Ct > 35)	- (Ct > 35)	+ or -	- (Ct > 35)	Invalid	Repeat extraction and qPCR for the failed sample.
- (Ct > 35)	+ (Ct < 35)	- (Ct > 35)	- (Ct > 35)	Invalid	Repeat qPCR for the failed sample with the failed master mix
+ (Ct < 35)	+ (Ct < 35)	- (Ct > 35)	- (Ct > 35)	Valid	One or multiple UTI pathogen(s) detected. The pathogen signal may out-compete the pathogen signals for qPCR.

Sample Results Example

Fluorefore	Positive Pathogen Detection
FAM+Cy5.5	
HEX+Cy5.5	
Texas Red+Cy5.5	
Multi detection	
NEC	
NC	
PC	

Performance

Detection Sensitivity

For LoD confirmation, 20 replicates of serial diluted samples were used for to confirm positive detection of UTI pathogens. Higher than 95% detection rate is required to confirm the LoD of the UTI pathogen testing.

Target	CFU/ml
Acinetobacter baumannii	2500 CFU/ml
Candida albicans	1250 CFU/ml
Candida glabrata	20000 CFU/ml
Candida tropicalis	20000 CFU/ml
Citrobacter freundii	156.25 CFU/ml
Enterobacter aerogenes	78.125 CFU/ml
Enterobacter cloacae	1250 CFU/ml
Enterococcus faecalis	625 CFU/ml
Enterococcus faecium	625 CFU/ml
Escherichia coli	39.06 CFU/ml
Klebsiella oxytoca	156.25 CFU/ml
Klebsiella pneumoniae	312.5 CFU/ml
Morganella morganii	78.125 CFU/ml
Mycoplasma hominis	625 CFU/ml
Proteus mirabilis	78.125 CFU/ml
Providencia stuartii	156.25 CFU/ml
Pseudomonas aeruginosa	1250 CFU/ml
Serratia marcescens	1250 CFU/ml
Staphylococcus aureus	625 CFU/ml
Staphylococcus saprophyticus	1250 CFU/ml
Streptococcus agalactiae	625 CFU/ml
Ureaplasma urealyticum	2.625 CFU/ml
Proteus vulgaris	462.5 CFU/ml

Cross-reactivity

Wet testing for the common UTI pathogens were used to assess cross-reactivity to preclude the occurrence of cross-reactivity or microbial interference with the assay. The wet testing was performed by spiking each UTI pathogen into negative urine sample to form 10 ^ 6 CFU/ml of each pathogen. The contrived samples then went through the DNA extraction and qPCR detection using LabTurbo AIO 48 system and Urinary Panel PCR reagents. No cross-reactivity was found.

Target	Positive Samples	Negative Samples
Acinetobacter baumannii	3/3	0/66
Candida albicans	3/3	0/66
Candida glabrata	3/3	0/66
Candida tropicalis	3/3	0/66
Citrobacter freundii	3/3	0/66
Enterobacter aerogenes	3/3	0/66
Enterobacter cloacae	3/3	0/66
Enterococcus faecalis	3/3	0/66
Enterococcus faecium	3/3	0/66
Escherichia coli	3/3	0/66
Klebsiella oxytoca	3/3	0/66
Klebsiella pneumoniae	3/3	0/66
Morganella morganii	3/3	0/66
Mycoplasma hominis	3/3	0/66
Proteus mirabilis	3/3	0/66
Proteus vulgaris	3/3	0/66
Providencia stuartii	3/3	0/66
Pseudomonas aeruginosa	3/3	0/66
Serratia marcescens	3/3	0/66
Staphylococcus aureus	3/3	0/66
Staphylococcus saprophyticus	3/3	0/66
Streptococcus agalactiae	3/3	0/66
Ureaplasma urealyticum	3/3	0/66

Interference Substance

An Interference Substance study was performed to assess the performance of the assay with common substances that could co-exist with UTI specimens. Contrived specimens were prepared by spiking the UTI pathogens into negative urine specimens to form specimens of 3x LoD. The contrived specimens were tested with interfering substances at relevant concentrations in triplicate. The interference substances used and the results are summarized in the tables below. All pathogens can be detected with the presence of common interference substances.

No.	Name	Concentration	Detection Rate
1	Monistat 1	3% W/V	100% for all targets
2	Monistat 7	3% W/V	100% for all targets
3	KY jelly classic	3% W/V	100% for all targets
4	Good Neighbor Pharmacy Feminine Wash	3% W/V	100% for all targets
5	Summer's Eve Extra Cleansing Vinegar & Water	3% V/V	100% for all targets
6	Vagisil Anti-Itch Vaginal Creme, Maximum Strength, 1 OZ	3% W/V	100% for all targets
7	Human blood	3% V/V	100% for all targets
8	BD Vacutainer C&S Preservative Urine Tube	Boric Acid 2.63 mg/ml, Sodium Borate 3.95 mg/ml, Sodium Formate 1.65 mg/ml	100% for all targets

Specimen and Testing Stability

The specimen and testing stability was performed to ensure consistent testing results can be obtained within a certain duration of time. Fifteen contrived samples including five positive samples at 4X LoD and five samples at 2X LoD were used for the specimen and testing stability evaluation. The samples were stored at 4 °C refrigerator and tested at Hour 0, Hour 24, Hour 72 and Hour 168 during the 7 day span. The LabTurbo AIO Urinary Panel PCR Detection Reagents can detect pathogens in urine specimens stored for up to 7 days.

Target	Hour 0	Hour 24	Hour 72	Hour 168
Acinetobacter baumannii	10/10	10/10	10/10	10/10
Candida albicans	10/10	10/10	10/10	10/10
Candida glabrata	10/10	10/10	10/10	10/10
Candida tropicalis	10/10	10/10	10/10	10/10
Citrobacter freundii	10/10	10/10	10/10	10/10
Enterobacter aerogenes	10/10	10/10	10/10	10/10
Enterobacter cloacae	10/10	10/10	10/10	10/10
Enterococcus faecalis	10/10	10/10	10/10	10/10
Enterococcus faecium	10/10	10/10	10/10	10/10
Escherichia coli	10/10	10/10	10/10	10/10
Klebsiella oxytoca	10/10	10/10	10/10	10/10
Klebsiella pneumoniae	10/10	10/10	10/10	10/10
Morganella morganii	10/10	10/10	10/10	10/10
Mycoplasma hominis	10/10	10/10	10/10	10/10
Proteus mirabilis	10/10	10/10	10/10	10/10
Proteus vulgaris	10/10	10/10	10/10	10/10
Providencia stuartii	10/10	10/10	10/10	10/10
Pseudomonas aeruginosa	10/10	10/10	10/10	10/10
Serratia marcescens	10/10	10/10	10/10	10/10
Staphylococcus aureus	10/10	10/10	10/10	10/10
Staphylococcus saprophyticus	10/10	10/10	10/10	10/10
Streptococcus agalactiae	10/10	10/10	10/10	10/10
Ureaplasma urealyticum	10/10	10/10	10/10	10/10

Clinical Comparison

For clinical comparison study, clinical UTI samples of confirmed detection results were processed by LabTurbo AIO Urinary PCR detection reagents to evaluate the conformity of the urinary pathogen identification.

Target	Conformity Rate
Candida albicans	96.67%
Candida glabrata	100.00%
Candida tropicalis	100.00%
Citrobacter freundii	100.00%
Enterobacter cloacae	100.00%
Enterococcus faecalis	90.00%
Escherichia coli	86.67%
Klebsiella oxytoca	90.00%
Klebsiella pneumoniae	93.33%
Morganella morganii	96.67%
Mycoplasma hominis	96.67%
Proteus mirabilis	90.00%
Providencia stuartii	93.33%
Pseudomonas aeruginosa	90.00%
Serratia marcescens	93.33%
Staphylococcus aureus	90.00%
Staphylococcus saprophyticus	100.00%
Streptococcus agalactiae	96.67%
Ureaplasma urealyticum	100.00%

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