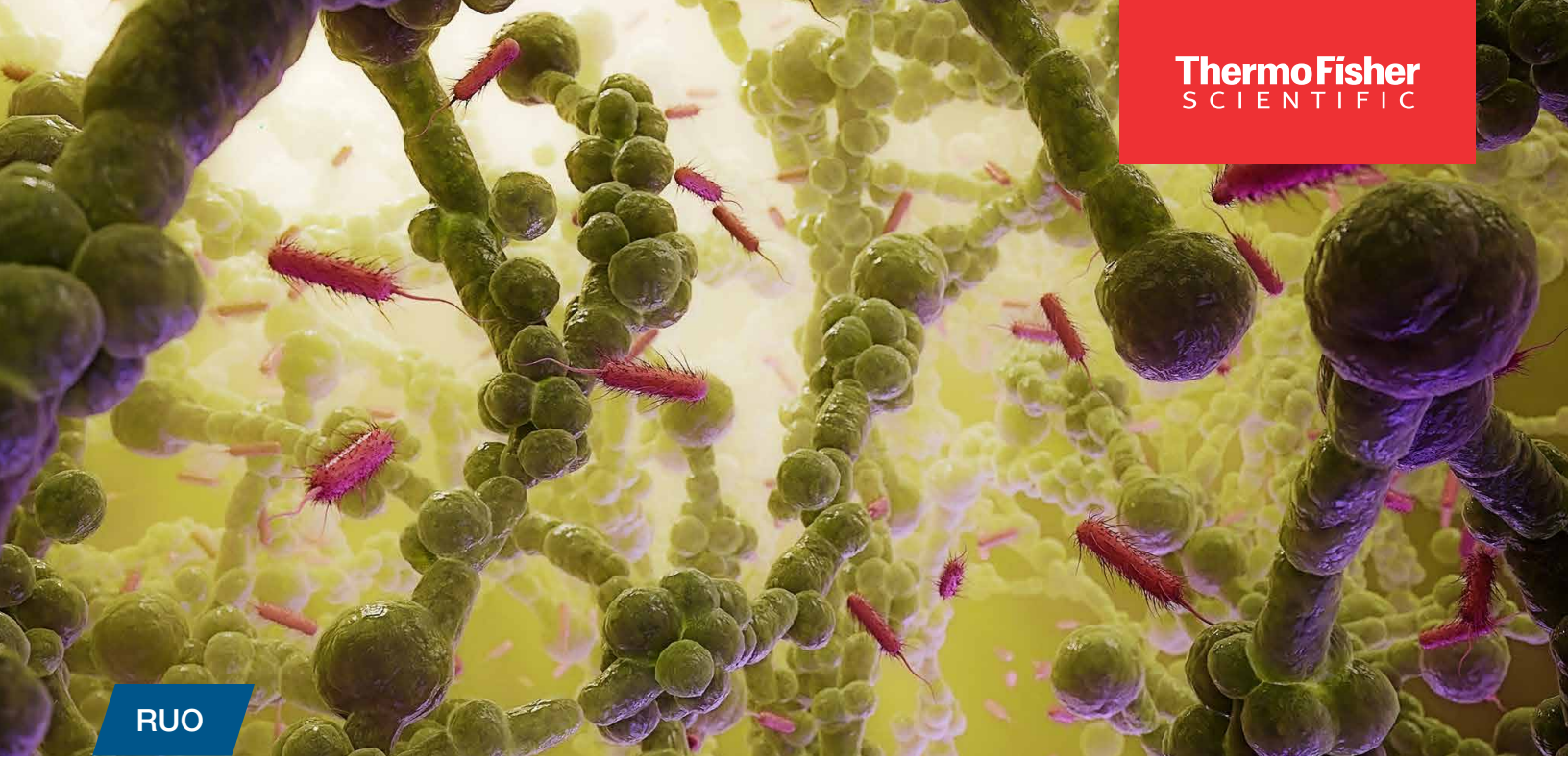


A complete workflow solution for detecting urinary tract infections using TrueMark Urinary Plus Panels

In this report, we show that:

- Applied Biosystems™ TrueMark™ Urinary Plus Panels for urinary tract infections meet rigorous performance criteria.
- Applied Biosystems TrueMark Urinary Plus Panels are convenient, duplexed Applied Biosystems™ TaqMan® assays that target a specific pathogen and an internal process control in each reaction, utilizing real-time PCR techniques. Assays are pre-spotted and dried-down on the plates.
- The Thermo Scientific™ KingFisher™ purification instruments, MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit, Applied Biosystems TrueMark Urinary Plus Panels, and Applied Biosystems™ QuantStudio™ real-time PCR Systems are part of a cost-effective, mid- to high-throughput solution with minimal hands-on time.
- Applied Biosystems TrueMark Urinary Plus Panels are available as three predefined panels in 96-well plate format, each targeting 8 pathogens and enabling testing of up to 12 samples per plate.
- Applied Biosystems TrueMark Urinary Panels are also available in custom options for 96-well or 384-well plate formats, ranging from 4 to 24 pathogens and up to 32 samples per plate.
- Applied Biosystems TrueMark Urinary Plus Panels are part of a portfolio of infectious disease panels that includes predefined TrueMark Respiratory Plus Panels and TrueMark STI, Vaginal, Lesion, and Genital Plus Panels, and these panels offer the flexibility to customize the panel assay content.

Introduction

Urinary tract infections (UTIs) are among the most prevalent diseases worldwide. UTIs not only impact the patient's quality of life but also create a significant clinical and economic burden[1]. Bacteria are the most common cause of UTIs, although fungi can also infect the urinary tract. Polymicrobial urinary colonization and infection are common and clinical research studies are needed to further understand the interplay and outcome of complex microbial communities[2].

To meet the need for more comprehensive coverage of urinary tract pathogens, we introduce a panel-based molecular research solution that includes a wide range of urinary tract pathogens. The TrueMark Urinary Plus Panels deliver a mid- to high-throughput, qPCR-based solution that can detect pathogenic organisms at very low concentrations. We offer three predefined TrueMark Urinary Plus Panels in 96-well plate format (Table 1). Each panel covers 8 pathogens and enables testing of up to 12 samples per plate. TaqMan assays are spotted and dried down in each well and only require the addition of master mix, extracted nucleic acid rfom samples. Each well contains a duplex reaction with both target and control assay. All panels utilize *Bacillus atrophaeus* as a spike-in process control. To provide maximal flexibility and to meet the needs of any laboratory, the

panel content can be customized and offered in 96- and 384-well plate formats.

This application note introduces the workflow for the TrueMark Urinary Plus Panels and demonstrates accurate performance in various tests for sensitivity and specificity.

Table 1. Predefined TrueMark Urinary Plus Panels

Applied Biosystems Panel	Pathogen Type	Pathogens Targeted	Internal Control*
TrueMark Urinary I Plus Panel (Cat. # A56288C)	Bacterial	*Pseudomonas aeruginosa* *Proteus mirabilis* *Escherichia coli* *Klebsiella pneumoniae* *Staphylococcus aureus* Coagulase-negative staphylococci *Staphylococcus saprophyticus*	*Bacillus Atrophaeus* spike-in control
	Fungi	*Candida albicans*	
TrueMark Urinary II Plus Panel (Cat. # A56289C)	Bacterial	*Corynebacterium riegelii* *Enterobacter aerogenes* *Aerococcus urinae* *Proteus vulgaris* *Acinetobacter baumannii* *Actinobaculum schaalii* *Enterococcus faecalis* *Streptococcus agalactiae*	
TrueMark Urinary III Plus Panel (Cat. # A56290C)	Bacterial	*Citrobacter freundii* *Enterobacter cloacae* *Enterococcus faecium* *Klebsiella oxytoca* *Serratia marcescens* *Mycoplasma hominis* *Morganella morganii* *Providencia stuartii*	

* Spike in the Applied Biosystems TaqMan Universal Extraction Control Organism (Cat. No. A39180) containing *Bacillus atrophaeus* for use as a process control.

For more information on the above predefined panels and the portfolio of options on TaqMan Array Plates, please view the **TrueMark Infectious Disease Research Solutions** flyer.

Materials and methods

Detection of urinary tract microbes using the TrueMark Urinary Plus Panels

TrueMark Urinary Plus Panels are designed to support mid- to high-throughput testing following the workflow depicted in Figure 1. For optimal performance, we recommend sample preparation and nucleic acid extraction using the MagMAX Viral/Pathogen Ultra Nucleic Acid Isolation Kit. *Bacillus atrophaeus* is utilized as a spike-in control at nucleic acid isolation of urine samples. Please refer to the User Guide on how to utilize the *Bacillus atrophaeus* spike-in control. Using the KingFisher purification instruments, automated extraction of up to 96 samples takes less than 60 minutes, with minimal hands-on time.

For more information on sample preparation recommendations, please view **MagMAX kits flyer**.

Setup of the PCR reactions is done by preparing nuclease-free water with the Applied Biosystems™ TrueMark™ Infectious Disease 1-Step Multiplex Master Mix (No ROX) before adding to the reaction wells containing dried-down assays*. Each TrueMark Urinary Plus Panel listed in Table 1 requires addition of the same sample to 8 defined wells (one well per target). To check for proper performance, we recommend running a Positive Control (PC) and Negative Extraction Control (NEC) in all experiments. We recommend using the Applied Biosystems™ TrueMark™ Amplification Control (Thermo Fisher Scientific Catalog Number A55698/A55699) as PC. Nuclease-free water can be used as NEC. Test results should be analyzed according to the analysis, interpretation, and QC parameters validated by your laboratory.

For more details on sample extraction, PCR setup, and running experiments, refer to the User Guide **"TrueMark™ Pathogen Assays on TaqMan™ Array Plates - User Guide"** (Pub. No. MAN0026671).

* The dried-down assays consist of primer and probes to amplify and detect the respective pathogen and *bacillus atrophaeus* as internal process control.

Receive samples	Prepare and purify samples	Prepare plate	Run PCR on real-time instrument	Analyze and report
Urine sample*	Use KingFisher systems and MagMAX kit	Combine sample with master mix and add to wells containing dried-down assay	Load plate into instrument and run PCR	QuantStudio Design and Analysis Software v2.6 or later

* Internally tested sample type.

Figure 1. Schematic workflow of TrueMark Urinary Plus Panels. The workflow shows extraction of nucleic acid containing virus and pathogens from samples using KingFisher purification instruments along with a MagMAX Nucleic Acid Isolation Kit, followed by preparation of the real-time PCR reaction. The run is performed in a QuantStudio real-time PCR instrument and analyzed using the Design and Analysis Software.

Results

High sensitivity and linear dynamic range of TrueMark Urinary Plus Panel assays

Synthetic templates were tested, and the results demonstrated high sensitivity of the TaqMan assays included in the TrueMark Urinary Plus Panels. We tested each target individually at a concentration of 10 copies/reaction. All testing was performed in triplicates.

Figure 2 shows that all TaqMan assays for urinary tract microbiota successfully amplified and detected their respective targets at a concentration of 10 copies/reaction with a Cq value below or around 35.

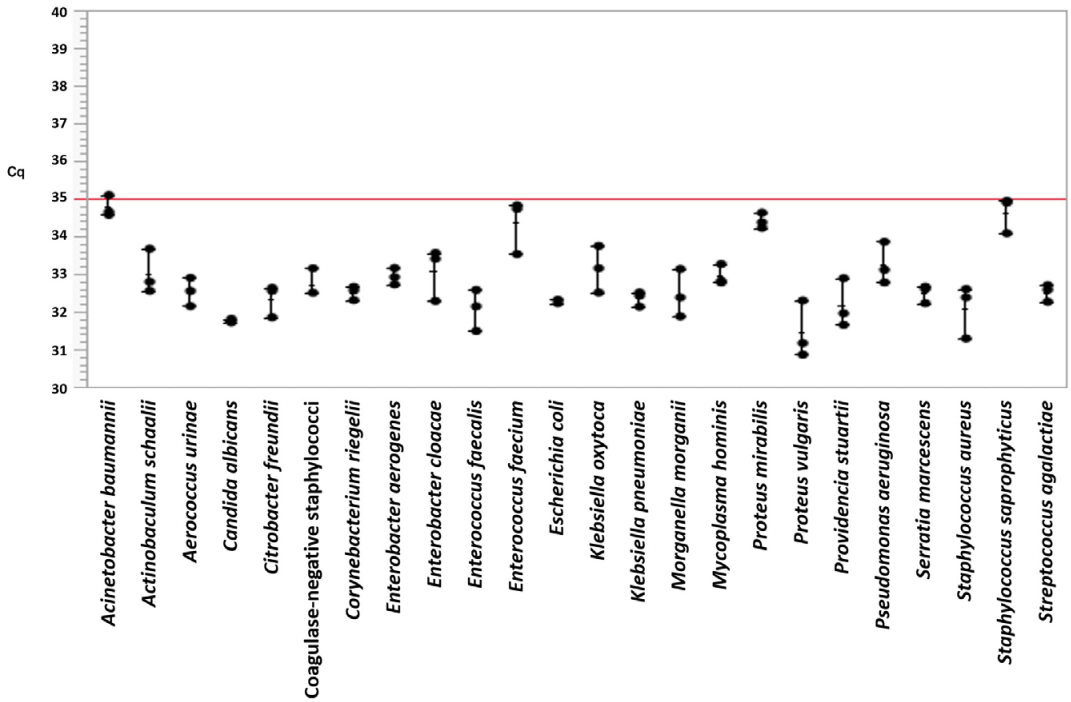
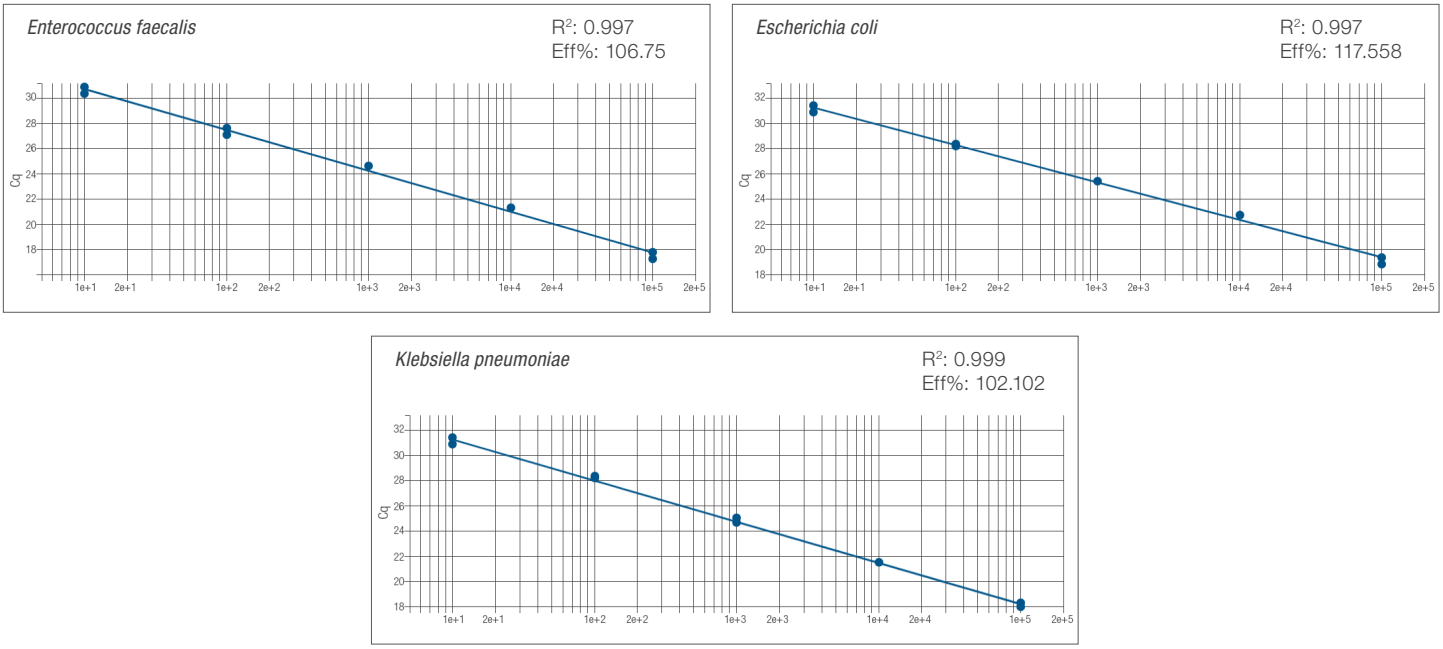


Figure 2. Analytical sensitivity of the TrueMark Urinary Plus Panel assays. Synthetic templates reflecting urinary tract microbiota targeted by the TrueMark Urinary Plus Panel assays were tested at 10 copies/reaction. Testing was performed on the QuantStudio 5 Real-Time PCR System (96-well, 0.2mL block) in triplicates, and threshold was set to 10,000.

To assess the linear dynamic range (LDR) of the TrueMark Urinary Plus Panel assays linear titration of synthetic templates for each pathogen were used at concentration ranging from 10^5 to 10 copies/reaction. Non-template controls (NTC) were also included (data not shown). All testing was performed in duplicates on the QuantStudio 5 Real-Time PCR System (96-well, 0.2mL block).

All assays demonstrated an LDR of at least 4 orders of magnitude (Figure 3). All assays showed R^2 greater than 0.98 and PCR efficiency close to 100%. As the TrueMark Urinary Plus Panel assays provide qualitative results, PCR efficiencies with greater than 110% do not impact result accuracy.

(A)



(B)

TaqMan Assay	R^2	Eff (%)	TaqMan Assay	R^2	Eff (%)	TaqMan Assay	R^2	Eff (%)
Escherichia coli	0.997	117.558	*Acinetobacter baumannii*	0.991	105.726	*Enterococcus faecium*	0.992	114.676
Klebsiella pneumoniae	0.999	102.102	*Proteus vulgaris*	0.997	97.979	*Klebsiella oxytoca*	0.994	111.877
Proteus mirabilis	0.995	110.140	*Actinobaculum schaalii*	0.996	103.489	*Morganella morganii*	0.995	103.502
Pseudomonas aeruginosa	0.997	104.394	*Staphylococcus saprophyticus*	0.996	111.434	*Mycoplasma hominis*	0.993	117.292
Staphylococcus aureus	0.995	104.777	Coagulase-negative Staphylococci	0.997	103.106	*Serratia marcescens*	0.997	112.173
Streptococcus agalactiae	0.998	111.757	*Aerococcus urinae*	0.998	104.600	*Providencia stuartii*	0.995	108.500
Candida albicans	0.999	108.016	*Corynebacterium riegelii*	0.999	106.739	*Citrobacter freundii*	0.997	107.291
Enterococcus faecalis	0.997	105.750	*Enterobacter aerogenes*	0.999	108.387	*Enterobacter cloacae*	0.996	118.377

Figure 3. Linear dynamic range results for TrueMark Urinary Plus Panel assays. LDR was calculated using concentration ranging from 10^5 to 10 copies/reaction. (A) Plots for 3 representative assays are shown (B) Representative R^2 and efficiency results for assays run on QuantStudio 5 Real-Time PCR Systems (96-well, 0.2mL block) are shown.

To demonstrate comparable LDR on different qPCR instrument platforms, we tested the performance of representative assays on the QuantStudio 5 Real-Time PCR System in 96-well and 384-well (0.1mL block) format. We also performed testing on the QuantStudio 5 Dx Real-Time PCR System (96-well, 0.2mL block)** and QuantStudio 7 Pro Real-Time PCR System (384-well, 0.1mL block). The synthetic templates for each pathogen were used at concentration ranging from 10^5 to 10 copies/reaction. Results are summarized in Table 2.

Table 2. Linear dynamic range results for different QuantStudio Real-Time PCR Systems

	QuantStudio 5 Real-Time PCR System (384-well, 0.1mL)		QuantStudio 7 Pro Real-Time PCR System (384-well, 0.1mL)		QuantStudio 5 Real-Time PCR System (96-well, 0.1mL)		QuantStudio 5 Dx Real-Time PCR System (96-well, 0.2mL)*	
TaqMan Assay	**R^2**	**Eff (%)**	**R^2**	**Eff (%)**	**R^2**	**Eff (%)**	**R^2**	**Eff (%)**
Acinetobacter baumannii	0.997	97.734	0.994	105.926	0.999	105.108	0.997	109.955
Candida albicans	0.995	105.129	0.995	99.466	0.957	92.987	0.991	100.083
Enterococcus faecium	0.999	100.179	0.998	99.496	0.991	104.898	0.998	102.638
Proteus mirabilis	0.998	97.882	0.998	99.237	0.996	105.548	0.997	94.811
Proteus vulgaris	0.995	99.747	0.997	102.092	0.996	104.232	0.999	102.979
Serratia marcescens	0.992	105.272	0.997	101.993	0.998	101.082	0.999	102.989
Staphylococcus aureus	0.995	96.745	0.998	106.65	0.99	102.03	1	104.229
Staphylococcus saprophyticus	0.999	101.079	0.996	99.992	0.996	109.125	0.998	101.356

* QuantStudio 5 Dx and QuantStudio 7 Pro Dx systems are For *In Vitro* Diagnostic Use. Test development mode is for Research Use Only. Not for use in diagnostic procedures.

The workflow sensitivity was determined using *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* as representative examples. Samples were prepared by spiking pathogens into negative human urine samples. Total nucleic acid of the target organisms was extracted using the MagMAX Viral/Pathogen Ultra Nucleic Acid Isolation Kit. The PCR was performed on the QuantStudio 5 Real-Time PCR System, 96-well, 0.2mL block. Table 3 depicts the lowest concentration that was detected in all replicates (10/10) with Cq values below 35.

Table 3. Workflow sensitivity for selected, representative pathogens

Target	Concentration*	Pathogen mean Cq (SD)	Hit Rate	RNase P mean Cq (SD)
Escherichia Coli	2 CFU/mL	31.92 (0.79)	10/10	25.36 (0.32)
Pseudomonas aeruginosa	100 CFU/mL	33.25 (0.37)	10/10	24.29 (0.21)
Candida albicans	100 CFU/mL	31.61 (0.59)	10/10	23.00 (0.23)

* CFU = Colony forming units

High specificity of TrueMark Urinary Plus Panel assays

All TrueMark Urinary Plus Panel assays have undergone rigorous bioinformatic analysis to help ensure maximum strain coverage while minimizing the potential for off-target cross-reactivity. Each assay was tested with on- and off-target genomic DNA isolated from target organisms. The inclusivity panel covered 23 urinary tract microbiota targets (Table 4). *Corynebacterium riegelii*, *Aerococcus urinae*, and *Actinobaculum schaalii* were not included in this panel.

Table 4. Urinary tract microbiota inclusivity controls

Organism type	Nucleic acid type	Organism	ATCC* Cat #
Bacteria	DNA	*Acinetobacter baumannii*	19606D-5
			BAA-1710D-5
		Citrobacter freundii	8090D
		Klebsiella aerogenes	13048DQ
		Enterobacter cloacae	13047D-5
		Enterococcus faecalis	700802DQ
		Enterococcus faecium	51559D-5
		Escherichia coli	BAA-460D-5
		Klebsiella oxytoca	700324D
		Klebsiella pneumoniae	700603DQ
		Morganella morganii	25830DQ
			35200D-5
		Mycoplasma hominis	23114D-5
		Proteus mirabilis	12453D-DQ
		Proteus vulgaris	29905DQ
		Pseudomonas aeruginosa	27853D-5
		Serratia marcescens	27137D-5
		Staphylococcus aureus	6538DQ
		Streptococcus agalactiae	BAA-1138D-5
		Staphylococcus saprophyticus	15305D-5
		Staphylococcus epidermidis	12228D-5
		Providencia stuartii	33672D
Fungi		*Candida albicans*	11006D-5

* American Type Culture Collection

Each organism was tested at ≥10^5 copies/reaction. The TrueMark Urinary Plus Panel assays provided highly specific results when screened simultaneously against the urinary tract microbial genomes (Table 5).

The coagulase-negative staphylococcus (CONS) assay cross reacts with *Staphylococcus aureus*. There may also be a minor cross-reactivity of the CONS assay with *Proteus mirabilis*. The CONS assay was also tested using additional *Proteus mirabilis* isolates and no cross reactivity was observed (data not shown). A similar result is observed for the *Serratia marcescens* assay, which shows cross-reactivity with one isolate of *Citrobacter freundi*, but not with others (data not shown). The *Enterobacter cloacae* assay cross reacts with *Citrobacter freundii*.

Note that CONS, *Staphylococcus aureus* and *Proteus mirabilis* assays are all included in the TrueMark Urinary I Plus Panel. By analyzing the results for all three assays, any cross-reactivity of the CONS assay can be assessed, and accurate sample results obtained. For example, samples positive for *Staphylococcus aureus* will be positive for the respective assay and the CONS assay while samples positive for Coagulase-negative staphylococci are only positive with the CONS assay. The *Enterobacter cloacae*, *Serratia marcescens* and *Citrobacter freundii* assays are all included in the TrueMark Urinary III Plus Panel. Analyzing results for all three assays allows researchers to accurately determine the sample status for each pathogen.

Table 5. Specificity testing of urinary tract microbiota assays

Pathogen \ Assay	*Pseudomonas aeruginosa*	*Streptococcus agalactiae*	*Proteus mirabilis*	PAN-*Candida*	*Enterococcus faecium*	*Escherichia coli*	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Enterobacter aerogenes*	*Proteus vulgaris*	*Acinetobacter baumannii*	Coagulase-negative staphylococci	*Staphylococcus saprophyticus*	*Citrobacter freundii*	*Enterobacter cloacae*	*Enterococcus faecalis*	*Klebsiella oxytoca*	*Serratia marcescens*	*Morganella morganii*	*Mycoplasma hominis*	*Providencia stuartii*
Pseudomonas aeruginosa	17.4																				
Streptococcus agalactiae		17.6																			
Proteus mirabilis			20.7									33.5									
Candida albicans				17.4																	
Enterococcus faecium					23.3																
Escherichia coli						22.8															
Klebsiella pneumoniae							17.8														
Staphylococcus aureus								17.7				20.5									
Klebsiella aerogenes									18.5												
Proteus vulgaris										16.3											
Acinetobacter baumannii											17.5										
Acinetobacter baumannii											17.4										
Staphylococcus epidermidis												20.7									
Staphylococcus saprophyticus												18.7	19.7								
Citrobacter freundii														17.3	31.1			32.9			
Enterobacter cloacae															17.3						
Enterococcus faecalis																17.5					
Klebsiella oxytoca																	18.9				
Serratia marcescens																		19.6			
Morganella morganii																			17.3		
Morganella morganii																			22.5		
Mycoplasma hominis																				19.0	
Providencia stuartii																					18.1

Note: To ensure correct results, custom panels including any of the three assays that may show cross-reactivity should include the respective counter assay(s) to allow accurate result interpretation.

CONS assay	*Staphylococcus aureus* assay	*Proteus mirabilis* assay	Pathogen detected
NEG	NEG	NEG	NEG
POS	NEG	NEG	Coagulase-negative *Staphylococcus*
POS	POS	NEG	*Staphylococcus aureus* assay*
POS/NEG	NEG	POS	*Proteus mirabilis***

* The sample contains *Staphylococcus aureus*. Results do not exclude dual infection that also contains CONS.
** The sample contains *Proteus mirabilis*. If CONS assay is positive, results do not exclude dual infection that also contains CONS.

Enterobacter cloacae assay	*Citrobacter freundii* assay	Pathogen detected
NEG	NEG	NEG
POS	NEG	*Enterobacter cloacae*
POS	POS	*Citrobacter freundii****

*** The sample contains *Citrobacter freundii*. Results do not exclude dual infection that also contains *Enterobacter cloacae*.

Testing of selected TrueMark Urinary Plus Panel assays against nontarget organisms in an exclusivity panel also demonstrated no cross-reactivity of the urinary tract microbiota assays with closely related species and other urinary microbes (Table 6 and data not shown).

Table 6. Urinary tract microbiota exclusivity controls

Organism type	Nucleic acid type	Organism	ATCC* Cat #
Bacteria	DNA	*Enterococcus hirae*	10541DQ
		Enterobacter nimipressuralis	9912D-5
		Escherichia fergusonii	35469D-5
		Aerococcus viridans	700406D-5
		Salmonella enterica	700720D-5
		Streptococcus mitis	49456D-5
		Pantoea agglomerans	33243D
Fungi		*Candida dubliniensis*	MYA-646DQ

* American Type Culture Collection

Conclusion

The TrueMark Urinary Plus Panel assays provides a reliable real-time PCR solution for identification of a broad range of urinary pathogens. The TrueMark Urinary Plus Panel assays demonstrated accurate performance in various tests for sensitivity and specificity.

The KingFisher purification instrument together with MagMAX Nucleic Acid Isolation Kits, offer an automated solution for extracting total nucleic acid that can be analyzed using the TrueMark Urinary Plus Panel assays.

The qualified TaqMan assays are available as predefined TrueMark Urinary Plus Panels, on 96-well plate format or can be included in customized panels and are offered in 96- and 384-well plate formats to support mid- to high-throughput testing. Together with the customer-friendly workflow, our TrueMark Urinary Plus Panel assay presents a low-cost solution for simultaneous detection of bacterial and fungal pathogens in urinary tract infections.

Ordering information

Description	Quantity	Cat. No
Other components required with TrueMark Urinary Custom Panels, sold separately		
TaqPath 1-Step Multiplex Master Mix (No ROX)	1 X 10mL	A28523
Other components recommended with TrueMark Urinary Plus and Custom Panels, sold separately		
KingFisher Apex Purification System	1 unit	5400930
KingFisher Flex Purification System	1 unit	A32681
MagMAX Viral/Pathogen Ultra Nucleic Acid Isolation Kit	100 preps	A42356
MagMAX Viral/Pathogen Nucleic Acid Isolation Kit*	Up to 200 preps	A42352
MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit*	Up to 2,000 preps	A48383R
MagMAX Viral/Pathogen Ultra Enzyme Mix	50 mL	A42366
TaqMan Universal Extraction Control Organism (*Bacillus atrophaeus*)	3 pellets	A39180
TrueMark Amplification Control (1 x 10^5 copies/µL)	1,000 µL	A55699
TrueMark Amplification Control (5 x 10^7 copies/µL)	50 µL	A55698
TrueMark Urinary Custom Panel offerings		
Truemark Urinary Panel	Varies	A55018
TrueMark Antibiotic Resistance Panel	Varies	A55021
TrueMark Custom Assay Panel	Varies	A55022
TrueMark Urinary Plus Panels, combo kit options		
Truemark Urinary I Plus Panel, Combo Kit	5 plates	A56288C
TrueMark Urinary II Plus Panel, Combo Kit	5 plates	A56289C
TrueMark Urinary III Plus Panel, Combo Kit	5 plates	A56290C
TrueMark Infectious Disease1-Step Multiplex Master Mix (No ROX)	3 x 1 mL	Included with Combo Kit

*Use with MagMaX Viral/Pathogen Ultra Enzyme Mix (A42366)

References

1. https://www.frontiersin.org/articles/10.3389/fpubh.2022.888205/full#B3
2. https://journals.asm.org/doi/full/10.1128/IAI.00652-20

Learn more at **thermofisher.com/truemarkpluspanels**