APPLICATION NOTE

High-throughput detection of UTI, STI, and wound pathogens using the SmartChip® Real-Time PCR System

- Detect more pathogens and antibiotic resistance genes with your customized panels
- Analyze up to 72 samples in one run, without the need for pre-amplification
- Add or remove targets based on detection needs, thanks to the flexibility of the SmartChip Real-Time PCR System

Introduction

Accurate identification of pathogenic microorganisms and antibiotic resistance genes is critical for public health. Urinary tract infections (UTIs) are a leading cause of morbidity and health-care expenditures, with a lifetime incidence of 50–60% in adult women (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6502976/). Sexually transmitted infections (STIs) and infected wounds also represent a significant burden on the health-care system.

Traditional culture-based techniques for detecting pathogens are slow, subjective, and lack sensitivity and specificity. Molecular techniques like PCR are increasingly used in the clinic to identify infectious organisms, permitting rapid identification of pathogens including slow-growing or difficult-to-cultivate microorganisms In addition, with the rise of multidrug-resistant organisms (https://www.nature.com/articles/s41467-022-29283-8), novel methods of identifying pathogens and antibiotic resistance genes (ARGs) are needed.

Our SmartChip Real-Time PCR System enables the throughput and sensitivity required by the next generation of infectious disease detection.

Each 5,184 nanowell chip enables:

- **Increased throughput.** A chip supports 14 different sample and assay configurations, enabling a run of 384 samples or assays with a run time of less than 4 hours. A total of 768 samples, with up to 4 replicates each, can be run in a single day.
- **Reduced hands-on time.** Performing the assay on our automated platform requires only 30 minutes of hands-on time.
- **Lower operating costs.** The reaction volume of each well on the chip is only 200 nl, lowering costs for master mixes and other reagents up to 200-fold.
- **Flexibility.** Create customized panels for combined pathogen tests.

In this application note, we showcase how our collaborator developed two SmartChip-based panels to detect pathogens and ARGs from UTI, sexually transmitted infection (STI), and wound infection samples. The panels detected more targets than similar products offered by leading competitors with comparable sensitivity, accuracy, specificity, and precision, providing researchers with an improved tool for advancing research on UTIs, STIs, and wound infections.

Assay development and flow

The SmartChip panels were tested for their ability to accurately detect common pathogens and ARGs associated with UTIs, STIs, and wound infections. The UTI panel includes 54 targets (pathogens and ARGs) associated with UTIs. The UTI + STI + wound panel includes the 54 targets of the UTI panel and an additional 18 targets associated with STIs and wound infections, for a total of 72 targets (see Table 1).

Total genomic DNA derived from cultured cells, whole-cell controls, and linearized oligo constructs containing cloned, PCR-amplifiable sequences specific to all targets were utilized in assays to determine the sensitivity, accuracy, and specificity of the SmartChip-based panels.

Nucleic acids were extracted from urine samples and contrived samples designed to mimic urogenital and wound swabs using commercially available DNA extraction and purification systems (explore Takara Bio's NucleoMag® Pathogen kits). Chip setup of the 54-target UTI panel and the 72-target UTI + STI + wound panel were performed using the SmartChip MyDesign Kit with functionally validated assays. SmartChip panels were run on the SmartChip Real-Time PCR Cycler (Figure 1).

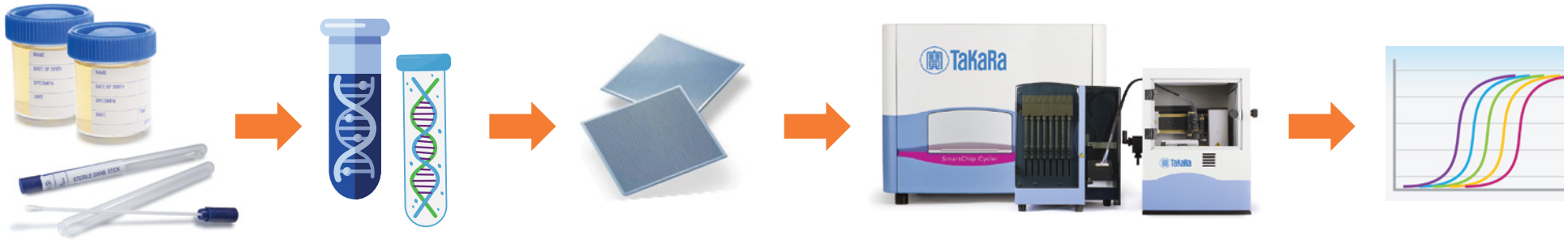


Figure 1. Workflow for detecting UTI, STI, wound pathogens, and ARGs using the Takara Bio SmartChip Real-Time PCR System.

The UTI and UTI + STI + wound panels cover more targets than other commercially available panels

The UTI panel covers common UTI pathogens including members of the *Acinetobacter*, *Candida*, *Citrobacter*, *Enterobacter*, and *Klebsiella* genera as well as *Escherichia coli* and several additional pathogens. Additionally, over 20 ARGs or ARG classes, including *ampC*, several OXA genes, multiple quorum sensing genes, multiple vancomycin resistance-conferring genes, and more are covered. Spike-in controls, including Xeno™, are included in this 54-target panel.

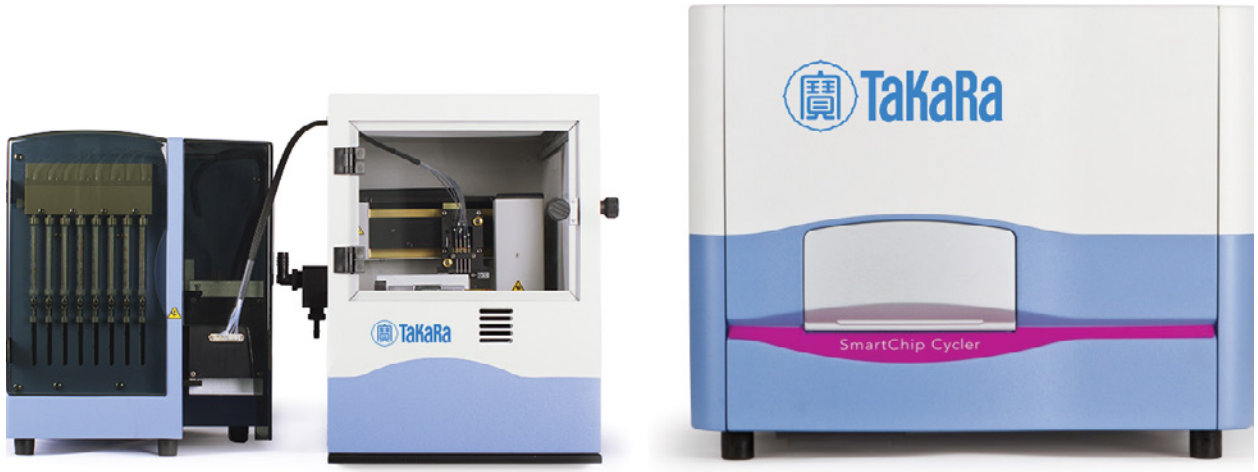
The 72-target UTI + STI + wound panel includes the 54 targets of the UTI panel with the addition of STI- and wound-specific pathogens and ARGs, including members of the *Mycoplasma*, *Neisseria*, *Chlamydia*, *Trichomonas*, *Treponema genera*, and genes conferring tetracycline resistance, erm genes, and several AAC genes. All 72 targets covered by the UTI + STI + wound SmartChip panel are listed in Table 1.

#	Target	#	Target	#	Target	#	Target
1	*Acinetobacter baumannii*	19	*Providencia stuartii*	37	*Candida glabrata*	55	OXA-1, GES
2	*Actinobaculum schaalii*	20	*Pseudomonas aeruginosa*	38	*Candida parapsilosis*	56	PER-1, PER-2
3	*Aerococcus urinae*	21	*Serratia marcescens*	39	*Trichomonas vaginalis*	57	mecA
4	*Citrobacter freundii*	22	*Staphylococcus aureus*	40	HSV1	58	vanA1, vanA2, vanB
5	*Citrobacter koseri*	23	*Streptococcus agalactiae*	41	HSV2	59	dfrA5, dfrA1
6	Coagulase Negative Staph	24	*Ureaplasma urealyticum*	42	IMP-1 group, IMP-16, IMP-7	60	Sul1, Sul2
7	*Corynebacterium riegelii*	25	Viridans Group Strep	43	OXA-23, OXA-72, OXA-40, blaOXA-48	61	nfsA
8	*Enterobacter aerogenes*	26	*Mycoplasma genitalium*	44	QnrA, QnrS, Qnr B	62	FOX
9	*Enterobacter cloacae*	27	*Haemophilus ducreyi*	45	Tet(M)	63	MOX/CMY
10	*Enterococcus faecalis*	28	*Treponema pallidum*	46	Mef(A)	64	BIL/LAT/CMY
11	*Enterococcus faecium*	29	*Neisseria gonorrhoeae*	47	AAC(6)-Ib (aac(6)-Ib),Ant(3),APH(3)-VIb (aph(3)-VI	65	KPC
12	*Escherichia coli*	30	*Chlamydia trachomatis*	48	ampC	66	erm(A)/erm(B)
13	*Klebsiella oxytoca*	31	*Bacteroides fragilis*	49	DHA	67	AAC(6)-Ib-cr (aac(6)-Ib-cr)
14	*Klebsiella pneumoniae*	32	*Kingella kingae*	50	ACC	68	TEM
15	*Morganella morganii*	33	*Bacillus atrophaeus*	51	SHV	69	16S
16	*Mycoplasma hominis*	34	*Candida dubliniensis*	52	VEB	70	RNaseP
17	*Proteus mirabilis*	35	*Candida albicans*	53	VIM	71	Xeno
18	*Proteus vulgaris*	36	*Candida auris*	54	CTX-M group 1, CTX-M group 2, CTX-M group 9, CTX-M group 8/25		

Table 1. Assay targets of the UTI + STI + wound panel on the SmartChip. The level of coverage provided by the UTI and UTI + STI + wound panels is superior to comparable panels, which usually include associated pathogens and not ARGs.

- **Bacterial**
- **Fungal**
- **Protozoan**
- **Viral**
- **ARG**
- **Controls**

The UTI and UTI + STI + wound panels offer sensitive pathogen detection



The UTI and UTI + STI + wound panels were tested for sensitivity by determining the lowest detectable DNA concentration that yielded positive results for the target pathogen(s) in approximately 95% of replicates. Synthetic oligos covering all pathogens in the panels were subjected to serial dilutions, resulting in an estimated limit of detection (LoD) between 100 and 1,000 copies/µl. The TaqMan Comprehensive Microbiota Control (CMC) was also used to confirm the LoD, with serial dilutions of 2,000, 1,000, 500, 200, and 100 copies/µl. The results showed that an LoD of 200 copies/µl was achieved for all pathogens except for *Enterobacter cloacae*, which had an LoD of 500 copies/µl. For detailed data, refer to the Appendix.

To ensure accurate pathogen detection at levels of 10^4–10^5 cells/ml, six different microorganism pools were introduced to normal urine or negative transport medium for wound and STI specimens at a concentration of 10^4 cfu/ml and tested using the mentioned panel-assays. The results showed that all pathogens were successfully detected from these samples. Additionally, repeating the assay on enumerated, whole-cell microorganisms resulted in the detection of all pathogens except for six microorganisms listed in the Table 2.

	Biological replicates	100 copies /µl		200 copies /µL		500 copies /µL		1,000 copies /µl		2,000 copies /µl	
Assay		Ct SD	Mean CT	Ct SD	Mean CT	Ct SD	Mean CT	Ct SD	Mean CT	Ct SD	Mean CT
Acinetobacter baumannii	7	0.42	32.21	0.33	31.03	0.24	29.40	0.09	28.54	0.07	27.51
Actinobaculum schaalii	7	0.34	31.84	0.34	30.74	0.17	29.33	0.20	28.41	0.05	27.38
Aerococcus urinae	7	0.46	31.35	0.24	30.57	0.23	29.28	0.11	28.18	0.08	27.15
Alloscardovia omnicolens	7	0.26	31.85	0.35	31.13	0.11	29.56	0.12	28.57	0.07	27.46
Bacillus atropheous	7	0.39	31.79	0.43	31.05	0.23	29.51	0.16	28.57	0.17	27.57
Candida albicans	7	0.20	30.99	0.50	30.39	0.13	28.83	0.08	27.85	0.05	26.85
Candida auris	7	0.53	32.58	0.45	32.10	0.24	30.80	0.10	29.61	0.13	28.64
Candida glabrata	7	0.28	32.01	0.39	31.02	0.13	29.65	0.16	28.69	0.11	27.63
Candida parapsilosis	7	0.33	32.24	0.13	30.90	0.24	29.67	0.11	28.67	0.09	27.60
Citrobacter freundii	7	0.41	31.86	0.48	31.05	0.16	29.55	0.11	28.45	0.04	27.43
Citrobacter koseri	7	0.33	31.24	0.45	30.33	0.15	28.90	0.14	28.07	0.09	27.06
Coagulase Negative Staph	7	0.33	31.56	0.13	30.59	0.04	29.48	0.05	28.48	0.04	27.39
Corynebacterium riegelii	7	0.35	31.45	0.17	30.35	0.12	29.01	0.15	28.04	0.07	26.96
Enterobacter aerogenes	7	0.32	31.74	0.50	30.84	0.23	29.57	0.14	28.62	0.09	27.49
Enterobacter cloacae	7	0.23	32.36	0.15	31.12	0.11	29.61	0.17	28.56	0.06	27.61
Enterococcus faecalis	7	0.30	31.31	0.21	30.59	0.08	29.16	0.04	28.15	0.08	27.24
Enterococcus faecium	7	0.40	33.07	0.40	32.55	0.16	29.16	0.13	30.06	0.12	28.99
Escherichia coli	7	0.15	32.00	0.20	30.52	0.16	30.99	0.07	28.26	0.05	27.40
Klebsiella oxytoca	7	0.35	31.52	0.43	30.70	0.12	29.36	0.11	28.31	0.07	27.40
Klebsiella pneumoniae	7	0.27	30.46	0.25	29.75	0.10	28.53	0.07	27.47	0.08	26.52
Morganella morganii	7	0.35	31.32	0.35	30.60	0.17	29.10	0.12	28.11	0.05	27.19
Mycoplasma hominis	7	0.46	33.05	0.50	31.68	0.13	30.52	0.04	29.40	0.10	28.47
Pantoea agglomerans	7	0.32	32.10	0.24	30.84	0.20	29.58	0.09	28.56	0.05	27.56
Proteus mirabilis	7	0.46	32.71	0.32	31.70	0.16	30.19	0.05	29.16	0.07	28.19
Proteus vulgaris	7	0.20	31.69	0.23	30.73	0.08	29.43	0.14	28.42	0.11	27.36
Providencia stuartii	7	0.34	32.51	0.44	31.65	0.41	30.27	0.15	29.41	0.04	28.17
Pseudomonas aeruginosa	7	0.22	32.00	0.16	31.17	0.11	29.79	0.08	28.79	0.09	27.85
Serratia marcescens	7	0.41	30.78	0.46	29.86	0.13	28.39	0.10	27.37	0.07	26.32
Staphylococcus aureus	7	0.19	31.98	0.16	30.95	0.10	29.46	0.06	28.50	0.08	27.47
Streptococcus agalactiae	7	0.39	32.06	0.27	31.27	0.10	29.86	0.09	28.80	0.13	27.86
Ureaplasma urealyticum	7	0.25	33.53	0.38	32.85	0.22	31.28	0.17	30.25	0.11	29.37
Viridans Group Strep	7	0.21	32.16	0.27	31.11	0.19	30.07	0.07	28.90	0.08	27.93
Grand total	7	0.38	31.90	0.37	30.99	0.21	29.61	0.14	28.60	0.09	27.59

Table 2. Confirmation of LoD was done by testing the panel against enumerated whole-cell organisms at levels of 10^4 to 10^6 cfu/ml.

The UTI and UTI + STI + wound panels ensure accurate pathogen detection

The accuracy of the UTI and UTI + STI + wound panels was determined by creating contrived samples and running those alongside verified positive and negative controls. For UTI samples, 29 negative urine samples were spiked with enumerated whole-cell controls (1 x 10^5 cfu/ml) and run alongside positive controls and negative urine samples. For STI and wound samples, 18 ESwab specimens in negative transport medium were spiked with enumerated whole-cell controls (1 x 10^5 cfu/ml) and run alongside positive and negative controls.

These assays revealed the panels could detect pathogens at clinically relevant levels with 100% accuracy. All contrived samples yielded positive results along with the positive control; similarly, no false positives were detected in the negative control samples (Table 3).

Assay	EC	ENTC	NEG_R1	NEG_R2	NEG_R3	NEG_R4	NEG_R5	NTC
Targets	**Average of Ct**	**Average of Ct**	**Average of Ct**	**Average of Ct**	**Average of Ct**	**Average of Ct**	**Average of Ct**	**Average of Ct**
Acinetobacter baumannii	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Bacillus atrophaeus	**21.11**	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Candida albicans	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Candida auris	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Candida glabrata	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Candida parapsilosis	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Citrobacter freundii	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Citrobacter koseri	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Coagulase Negative Staph	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Corynebacterium riegelii	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Enterobacter aerogenes	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Enterobacter cloacae	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Enterococcus faecalis	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Enterococcus faecium	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Escherichia coli	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Klebsiella oxytoca	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Klebsiella pneumoniae	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Morganella morganii	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Mycoplasma hominis	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Pantoea agglomerans	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Proteus mirabilis	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Proteus vulgaris	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Providencia stuartii	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Pseudomonas aeruginosa	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Serratia marcescens	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Staphylococcus aureus	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Streptococcus agalactiae	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Ureaplasma urealyticum	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Viridans Group Strep	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Xeno	Neg	Neg	**27.49**	**27.51**	**27.36**	**27.34**		

Table 3. The table outlines the data obtained to show that there are no false positive results for negative specimens. These samples were run along with positive-control and contrived-positive specimens, ruling out the possibility of cross-contamination between specimens during the loading process. Also, the extraction control (*B. atrophaeus*) shows that the enzyme digestion and extraction processes were performed properly.

The UTI and UTI + STI + wound panels are highly specific to infectious pathogens

To confirm the specificity of the panels, exclusivity panels consisting of genomic DNA derived from a collection of non-pathogenic, near-neighbor microorganisms were run with the Taqman pathogen-detecting primer sets used for the SmartChip panels.

Four extracted DNA pools were acquired from the laboratory, diluted to a concentration of 10^5 copies/µl, and run in triplicate. No cross-reactivity or false positive calls were detected for any of the targets covered by the SmartChip UTI panel (Table 4).

Reproducible pathogen and ARG detection

The reliability and reproducibility of the SmartChip protocol for UTI, STI, and wound pathogen and ARG detection were assessed via a series of experiments testing technical variability (data not shown). Two different operators performed triplicate reactions using CMC at 10^5 copies/µl, yielding 100% concordance across all six reactions. Similarly, three contrived urine and ESwab specimens spiked with enumerated whole cells (10^5 cells/ml) were run in triplicate in one run, yielding expected results. These results demonstrate that the panels can be used for reliable, reproducible detection of key pathogens and ARGs.

Microorganism	Pool/ Tube #	Final conc in pools (copies/µl)	% positive
Acinetobacter bereziniae	1	1.00E+05	0
Raoultella planticola		1.00E+05	
Proteus penneri		1.00E+05	
Proteus hauseri	2	1.00E+05	0
Moellerella wisconsensis		1.00E+05	
Brenneria salicis		1.00E+05	
Pantoea agglomerans	3	1.00E+05	0
Corynebacterium glucuronolyticum		1.00E+05	
Pseudomonas syringae		1.00E+05	
Enterococcus hirae	4	1.00E+05	0
Hafnia alvei		1.00E+05	
Salmonella enterica		1.00E+05	
Candida dubliniensis		1.00E+05	

Table 4. Detection of pathogens in urine, wound swab, and urogenital swab specimens using analytically validated assays loaded in nanoscale SmartChip plate to test for any cross-reactivity.

Conclusions

- SmartChip based UTI and UTI + STI + wound panels yield consistent, reproducible, sensitive, accurate, and specific detection of pathogens and antibiotic resistance genes from urine, urogenital, and wound swabs.
- Performance was validated using a series of contrived samples and whole cells at relevant concentrations with verified positive and negative controls.
- An industry-unequaled 54 targets were detected with 100% specificity using the UTI panel and 72 targets with the UTI + STI + wound panel.
- Takara Bio's SmartChip Real-Time PCR System allows for a rapid, low-cost solution for detecting pathogens and antibiotic resistance genes.

PRODUCTS

Cat. #	Product	Size
640022	SmartChip Real-Time PCR System	Each
638349	5X PrimePath™ Probe qPCR Kit, no ROX, GPR	200 Rxns
638347	5X PrimePath Probe qPCR Kit, no ROX, GPR	1,000 Rxns
640032	SmartChip MyDesign Kit (430-000110)	1 chip
640036	SmartChip MyDesign Kit (430-000244)	20/Pack
640018	MSND 384-Well Source Plate and Seals (430-000025)	20/Pack
640037	MSND 384-well Source Plate and Seals (430-000258)	120/Pack
744210.4	NucleoMag Pathogen	4 x 96 Preps

Get in touch

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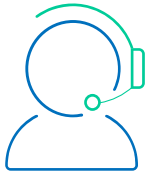

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