A high-throughput qPCR assay panel for rapid detection of pathogens in urinary tract infections and beyond

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Introduction

Urinary tract infections (UTIs), caused by a range of bacterial and fungal microorganisms, are common and painful conditions affecting millions of people worldwide. Sexually transmitted infections (STIs) and infected wounds also represent a significant burden on the healthcare system. These pathogens are usually detected with urine dipstick or culture. However, these assays are limited by their inability to detect slow-growing or difficult-to-culture pathogens. To address this, we developed a sensitive and specific qPCR-based panel for the identification of these types of pathogens on the high-throughput SmartChip® ND™ Real-Time PCR System. This system is able to perform 5,184 massively parallel, singleplex qPCR reactions at nanoliter scale across multiple samples and reactions.

1 UTI, STI, and wound panel targets

Panel targets			
Acinetobacter baumannii	*Pseudomonas aeruginosa*	blaMOX	*Candida dubliniensis*
Actinotignum schaalii	*Serratia marcescens*	blaOXA-1	*Chlamydia trachomatis*
Aerococcus urinae	*Staphylococcus aureus*	blaOXA-23	*Haemophilus ducreyi*
Candida albicans	*Staphylococcus epidermidis*	blaOXA-40	*Herpes simplex virus type 1*
Candida auris	*Staphylococcus haemolyticus*	blaOXA-48	*Herpes simplex virus type 2*
Candida glabrata	*Staphylococcus saprophyticus*	blaOXA-72	*Mycoplasma genitalium*
Candida parapsilosis	*Streptococcus agalactiae*	blaPER-1	*Neisseria gonorrhoeae*
Candida tropicalis	*Streptococcus anginosus*	blaPER-2	*Treponema pallidum*
Citrobacter freundii	*Streptococcus oralis*	blaSHV	*Trichomonas vaginalis*
Citrobacter koseri	*Ureaplasma urealyticum*	blaTEM	*Bacteroides fragilis*
Coagulase-negative staphylococci (CoNS)	blaACC	blaVEB	*Kingella kingae*
Corynebacterium riegelii	blaACT	blaVIM	*Streptococcus pyogenes*
Enterobacter cloacae	blaCMY	dfrA1	AAC(6')-Ib
Enterococcus faecalis	blaCTX-M 1	dfrA5	AAC(6')-Ib-cr
Enterococcus faecium	blaCTX-M 2	mecA	ANT(3")-IIa
Escherichia coli	blaCTX-M 8	nfsA	APH(3')-VIa
Klebsiella aerogenes	blaCTX-M 9	QnrA	ermA
Klebsiella oxytoca	blaDHA	QnrB	ermB
Klebsiella pneumoniae	blaFOX	QnrS	mefA
Metamycoplasma hominis	blaGES	sul1	tetM
Morganella morganii	blaIMP-1	sul2	*Bacillus atrophaeus*
Proteus mirabilis	blaIMP-16	vanA	16S
Proteus vulgaris	blaIMP-7	vanB	RNaseP
Providencia stuartii	blaKPC	vanC	Spike-in control

Figure 1. Target organisms and antimicrobial genes list for the UTI, STI, and wound panel. The blue text represents UTI targets, the orange text represents STI targets, the green text represents wound infection targets, and the purple text represents controls.

2 In silico design: overview

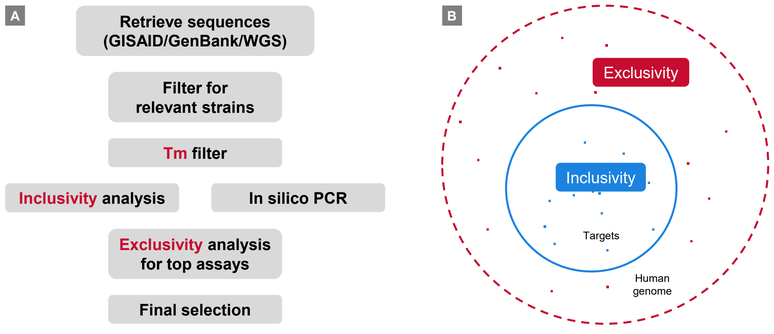

Figure 2. Primer and probe selection process. Panel A. The primer and probe selection process consists of the following steps: (1) retrieval of relevant strain information from databases; (2) filtering of strains based on clade and date of collection; (3) filtering the assays based on alignment, encompassing strain-inclusivity, exclusivity, and other qPCR design criteria; and (4) selection of the final forward/reverse primers and FAM-labeled probes for lab testing. **Panel B.** Graphical representation of filtering primers and probes based on inclusivity for a desired strain and exclusivity of undesired amplification from strains present in the sample mixture.

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3 SmartChip ND high-density qPCR workflow

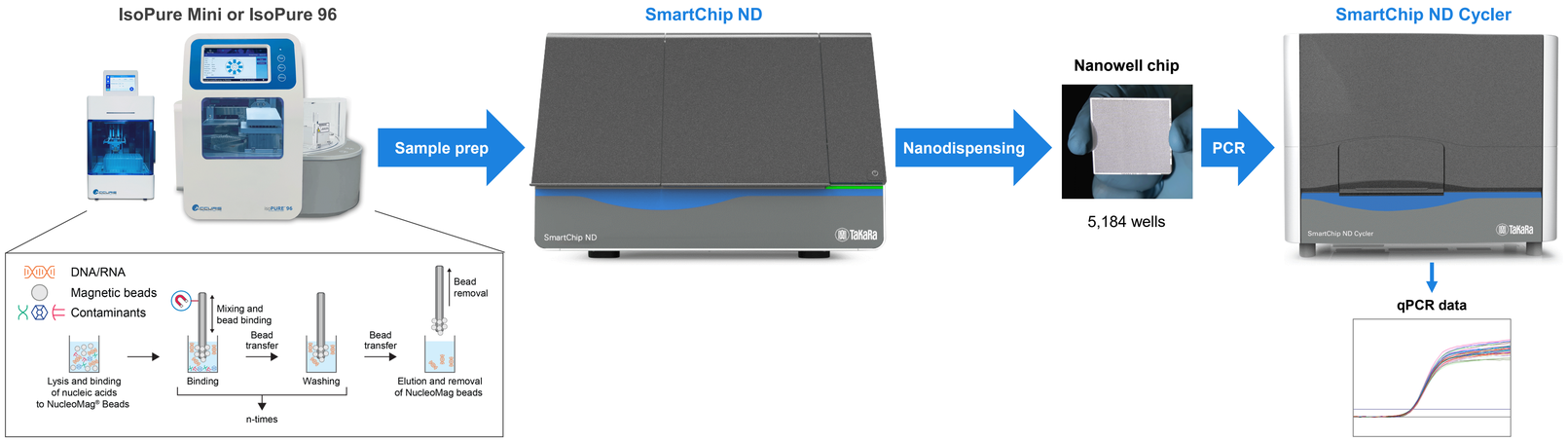

Figure 3. The SmartChip ND workflow. First, DNA from samples is extracted using an IsoPure Mini or IsoPure 96 instrument. Then, sample DNA is dispensed into a 5,184 nanowell chip using the SmartChip Nanodispenser (ND). In the last step, RT-PCR cycling and analysis are performed on the SmartChip ND Cycler.

4 SmartChip ND system uniformity

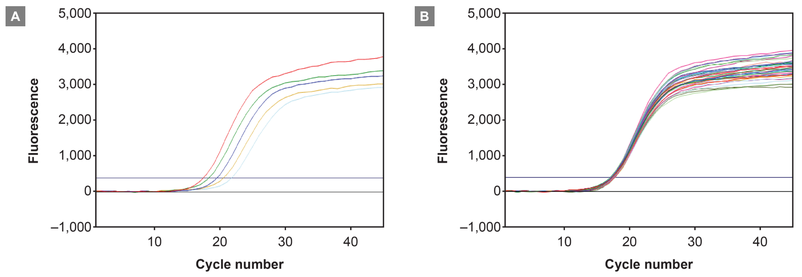

Figure 4. Assay uniformity of the SmartChip ND workflow. Panel A. The amplification curves of a representative qPCR assay for one replicate across a five-point two-fold dilution series. **Panel B.** The same representative assay for 36 replicates. The Cq values show very tight convergence for all the assays with very low variability. The coefficient of variance (CV) of the assay at the lowest copy number tested (200 copies/reaction) was ≤1%.

6 UTI, STI, and wound panel: assay linearity

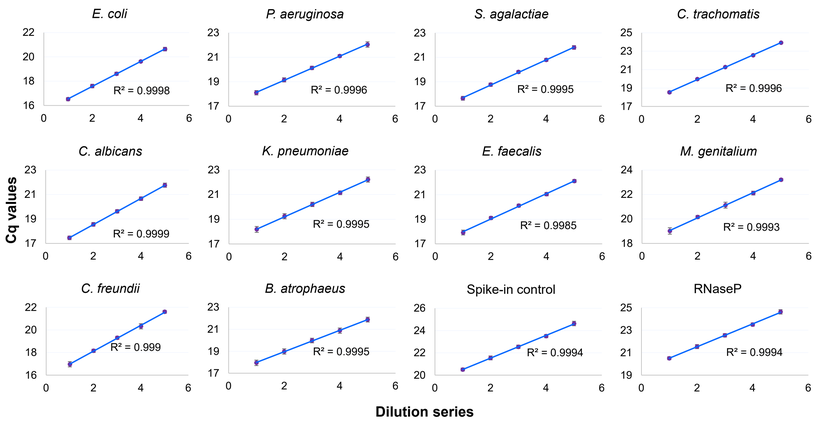

Figure 6. Linearity curves show the efficacy of the assays and accuracy of the SmartChip ND dispenses. Select assays in the UTI, STI panel, and wound panel were tested across a five-point two-fold dilution series, ranging from 1,000–16,000 copies per reaction. More than three automated replicates were run for each dilution. Excellent linearity (high R^2 values) between concentration and Cq values demonstrates accurate and reproducible dispensing.

5 Variability of the SmartChip ND system is comparable to plate-based systems

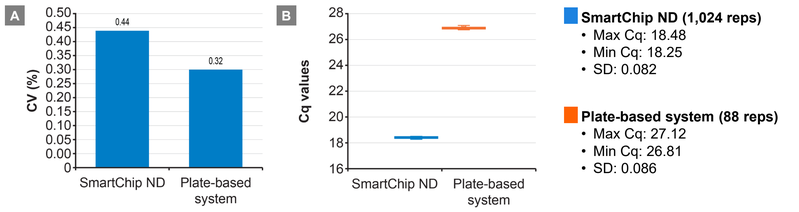

Figure 5. The SmartChip ND system exhibits a level of variability comparable to plate-based real-time PCR systems. Panel A. The coefficient of variance (CV) of a representative assay performed on the SmartChip ND Cycler and a plate-based, real-time PCR system. **Panel B.** The Cq of reactions run on the SmartChip ND Cycler is lower than those run on the plate-based PCR system using the same amount of starting template. Because of the nanoliter reaction size, the threshold for Cq detection is reached at an earlier cycle as the effective concentration can be reached more quickly.

7 SmartChip ND throughput possibilities

SmartChip panel capacities			
Samples	**Assays**	**Replicates**	**Example panels**
24	72	Triplicate	Urinary tract infection
32	48	Triplicate	Wound infection
72	24	Triplicate	Sexually-transmitted infection
216	12	Duplicate	Respiratory viral infection

Figure 7. Some of the possible combinations of samples and assays on the SmartChip ND system. Nanoliter-scale reactions can dramatically cut reagent costs.

Conclusions

- We successfully developed a combined assay panel for UTI, STI, and wound infections, comprising over 80 hydrolysis probe-based assays.
- This comprehensive panel includes controls, common antimicrobial resistance (AMR) genes, and targets for bacterial, fungal, and other pathogenic species.
- The 5,184-well SmartChip ND Real-Time PCR System proved exceptionally well-suited for this application, offering high sensitivity, high sample throughput, and panel flexibility.
- Our study demonstrates the successful implementation of a large pathogen detection qPCR assay panel capable of detecting common bacterial and fungal pathogens with a high-density qPCR system.

Meeting details and poster download:
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