Poster # ID031

Comprehensive UTI assay panel utilizing exogenous DNA spike-in control for improved detection accuracy

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Introduction

Why a comprehensive UTI panel? There is a growing need for molecular research products with high sensitivity and specificity that can detect a broad range of pathogens including viruses, bacteria, and fungi that are common in the urinary tract and in wound specimens. Traditional detection methods like urine dipsticks and cultures often miss hard-to-culture pathogens. Molecular methods are faster and less prone to contamination but need comprehensive panels to identify rare species and antibiotic resistance genes. To address this, we developed a sensitive qPCR-based panel for accurate UTI detection. Our assay design is based on cross-reactivity data against almost all known human pathogens collected from major databases, such as GISAID, GenBank, and WGS. This comprehensive molecular approach—integrated with Research Use Only (RUO) high-throughput technology like the SmartChip® platform—significantly enhances detection precision. This method ensures more accurate results and effectively addresses challenges posed by antibiotic resistance genes (ARGs) and pathogen diversity, making it a robust solution for pathogen detection.

Spike-in control

Extraction

Amplification

Detection

Why a spike-in control? To enhance qPCR quality control, introducing a spike-in control with a unique or exogenous DNA sequence helps address the shortcomings of existing panels, particularly in preventing false negatives. Added during the sample lysis step, it acts as both a qPCR and process control, ensuring procedural consistency and reliable bacterial load quantification. This control monitors the entire qPCR process, detects inhibitors, normalizes data, and aids in troubleshooting, ultimately improving the detection precision and overall value of the assay panel.

Methods

We developed a targeted 96-assay panel to detect pathogens common in UTIs, STIs, and wound infections, along with the relevant antimicrobial resistance genes. The primer and probe selection process involved several key steps:

1. **Data retrieval:** relevant strain information was collected from databases such as GISAID, GenBank, and whole-genome sequencing (WGS) repositories
2. **Strain filtering:** strains were filtered based on clade classification and date of collection to ensure up-to-date and relevant coverage
3. **Assay filtering:** assays were evaluated based on sequence alignment to guarantee strain inclusivity, exclusivity, and adherence to other qPCR design criteria, thus preventing non-specific amplification
4. **Primer and probe selection:** final forward and reverse primers, along with FAM-labeled probes, were selected for laboratory testing

The assays designed in silico were validated using positive controls. To ensure procedural reliability, an exogenous DNA sequence was spiked into each sample during the lysis step of DNA extraction, serving as a workflow control. Linearity was assessed using ten-fold serial dilutions, calculating R^2 values and PCR efficiencies (E) from the standard curves. All assays were validated in duplex with the spike-in control (in HEX channel), confirming specificity with positive and negative controls. This spike-in control functioned as both a qPCR and process control, ensuring the reliability of the entire procedure. After determining the limit of detection (LoD), the assays were confirmed to have an LoD of 20 copies per reaction or better.

1 Comprehensive UTI combo panel

UTI

Acinetobacter baumannii
Actinobaculum schaalii
Aerococcus urinae
Bacillus atrophaeus
Candida albicans
Candida auris
Candida glabrata
Candida parapsilosis
Candida tropicalis
Citrobacter freundii
Citrobacter koseri
Corynebacterium riegelii
Enterobacter aerogenes
Enterobacter cloacae
Enterococcus faecalis
Enterococcus faecium
Escherichia coli
Klebsiella oxytoca
Klebsiella pneumoniae
Morganella morganii
Mycoplasma hominis
Proteus mirabilis
Proteus vulgaris
Providencia stuartii
Pseudomonas aeruginosa
Serratia marcescens
Staphylococcus aureus
Staphylococcus epidermidis
Staphylococcus haemolyticus
Staphylococcus lugdunensis
Staphylococcus saprophyticus
Streptococcus agalactiae
Streptococcus anginos
Streptococcus oralis
Ureaplasma urealyticum
blaACC
blaACT/blaMIR
blaCMY
blaCTX-M 1
blaCTX-M 2
blaCTX-M 8/25
blaCTX-M 9
blaDHA
blaFOX
blaGES
blaIMP-1
blaIMP-7
blaIMP-16
blaKPC
blaMOX
blaOXA-1
blaOXA-23
blaOXA-40
blaOXA-48
blaOXA-72
blaPER-1
blaPER-2
blaSHV
blaTEM
blaVEB
blaVIM
dfrA1
dfrA5
mecA
nfsA
QnrA
QnrB
QnrS
sul1
sul2
vanA
vanB
vanC
Alien
RNaseP
16s

Wound

1. *Bacteroides fragilis*
2. *Kingella kingae*
3. *Streptococcus pyogenes*
4. AAC (6')-Ib
5. AAC (6')-Ib-cr
6. ANT (3")-IIa/aadA
7. APH (3')-VIa
8. ermA
9. ermB
10. mefA
11. tetM

STI

1. *Candida dubliniensis*
2. *Chlamydia trachomatis*
3. *Haemophilus ducreyi*
4. HSVI
5. HSV2
6. *Mycoplasma genitalium*
7. *Neisseria gonorrhoeae*
8. *Treponema pallidum*
9. *Trichomonas vaginalis*

Legend: Fungus Parasite Virus Bacteria Antibiotic resistance gene Control

Figure 1. List of target organisms and antibiotic resistance genes for the UTI, STI, and wound panel. There are 72 UTI targets, 9 STI targets, 11 wound infection targets, and 4 controls. The detection of antimicrobial resistance (AMR) genes in UTI and STI samples is a common focus of ongoing research. Identifying these AMR genes provides insight into whether the pathogen carries resistance to certain antibiotics, which is useful for detecting antibiotic-resistant strains.

2 SmartChip ND™ (SCND) workflow

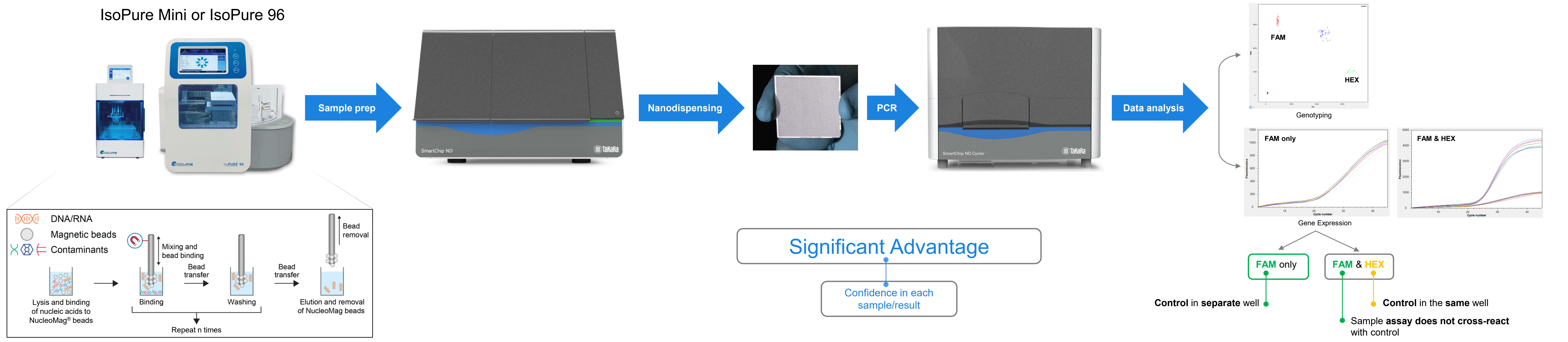

Figure 2. The SmartChip ND workflow for highly reliable and consistent results. First, DNA is extracted from samples using the IsoPure Mini or IsoPure 96 instrument. During this step, an exogenous spike-in control is added to the sample. The extracted DNA is then dispensed into a 5,184-nanowell chip using the SmartChip Nanodispenser. RT-PCR cycling and analysis are conducted on the SmartChip ND Cycler. The spike-in control and option for duplexing provide quality checks that ensure reliable results for every sample tested.

3 Linearity data demonstrates assay efficacy and accuracy of SmartChip ND dispensing

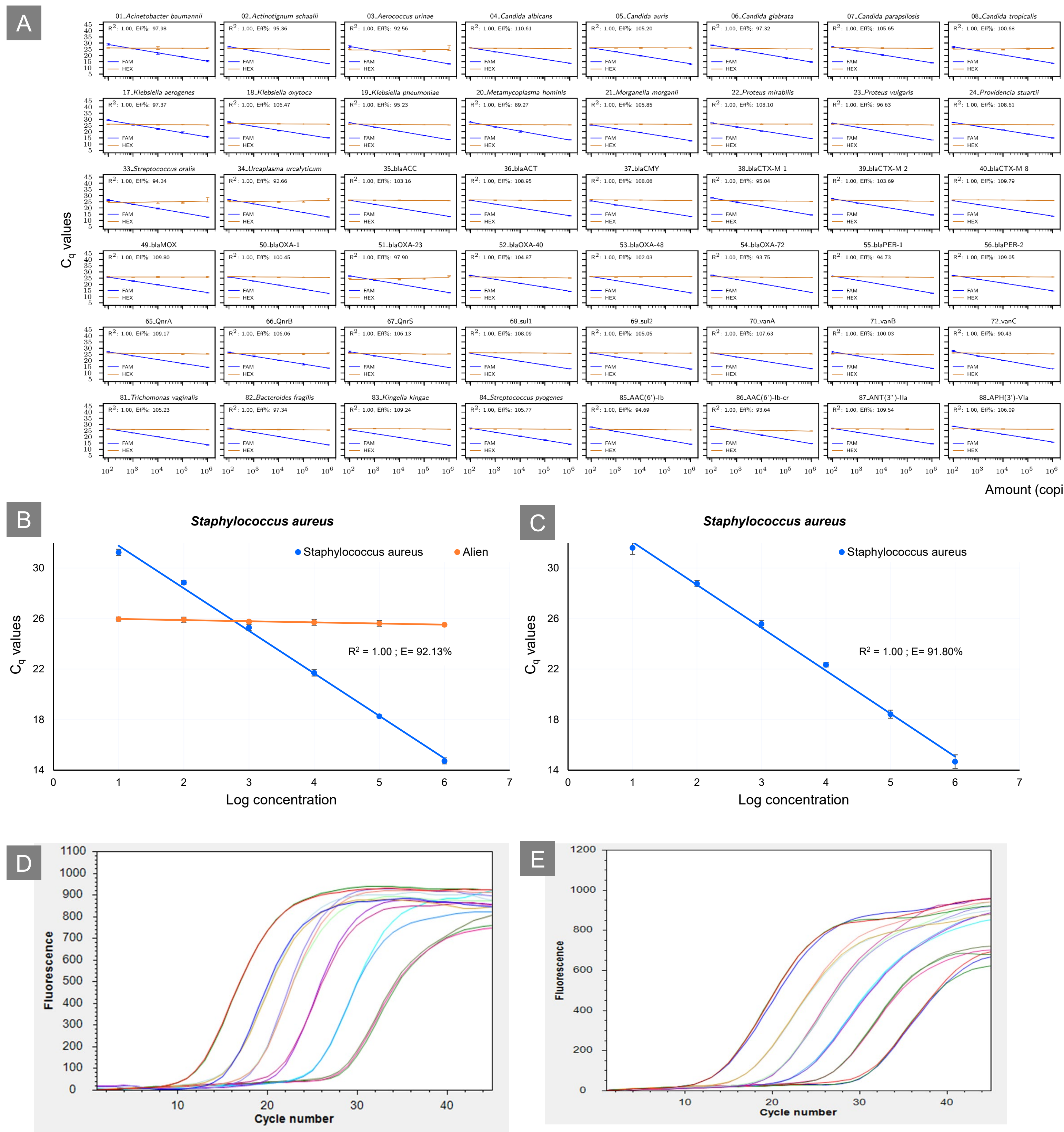

Figure 3. Linearity data demonsrates assay efficacy and accuracy of SmartChip ND dispensing. Panel A. Linearity for all 95 assays in duplex. Enlarged image of a single example in duplex (**Panel B**) and in singleplex (**Panel C**). Example of amplification curve for *Staphylococcus aureus* (**Panel D**) and for *Aerococcus urinae* (**Panel E**). All assays were tested in duplex as well as singleplex format (only one example shown) with identical copies of exogenous spike-in DNA controls added to varying dilutions of the analytical UTI control. The UTI, STI, and wound panel assays were evaluated across a dilution series ranging from 1×10^1 to 1×10^6 copies per reaction, with over three automated replicates per point. The results demonstrated excellent linearity (R^2 >0.99) between concentration and C_q values, confirming accurate and reproducible sample dispensing from the SmartChip ND instrument. The negligible difference in C_q values between singleplex and duplex formats demonstrates minimal cross-talk between assays on the SmartChip Cycler, highlighting its reliable performance.

4 SmartChip ND reactions compared to traditional plate-based qPCR reactions

Reagents and time required for 5,184 rxn (72 x 72 well layout) run

Category	Plate qPCR (10 µl rxn)	SCND	Fold difference
Master mix	>30 ml	<0.5 ml	>60
Assay mix	>3 ml	~100 µl	>30
Turn around time	~25 hr	~4 hr	>6

Figure 4. Plate-based qPCR reactions vs. SmartChip ND reactions. A traditional plate-based qPCR system employing a 384-well format with a 10 µl reaction volume is contrasted with a single SmartChip run utilizing a 72 x 72 well layout. Switching to the high-throughput SCND platform results in a significant reduction in reaction volumes, which provides substantial reagent savings. Additionally, the SCND system can process a much higher number of samples per run (5,184 wells per chip), making it more than six times faster than plate-based qPCR which saves valuable time.

Conclusions

- A comprehensive 96-assay qPCR panel simplifies the detection of pathogens common in UTIs, STIs, and wound infections, and identifies associated antibiotic resistance genes.
- All assays in the UTI panel have an analytical LoD value of 20 copies per reaction or better, with some as low as 10 copies per reaction.
- A spike-in control ensures qPCR accuracy by detecting inhibitors and preventing false negatives.
- The SmartChip ND platform offers excellent accuracy, minimal cross-talk, and significant time and cost savings, with the capacity to run 5,184 reactions in a single run.

Meeting details and poster download:
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