Direct qPCR amplification from human urine samples

Abstract

Quantitative PCR (qPCR) workflows typically require an extraction step to collect nucleic acids and remove PCR inhibitors from crude biological samples, such as human urine, prior to amplification. In this technical note we will introduce you to IDT's PrimeTime™ One-Step 4X Broad-Range Master Mix as a ready-to-use alternative to costly and time-consuming extraction steps. Our data demonstrate that extraction-free qPCR amplification can efficiently amplify from pooled crude biological samples when compared to synthetic controls. Additionally, when amplifying from research samples positive for bacteria associated with urinary tract infections (UTIs), extraction-free amplification produced similar or improved results compared to qPCR using samples extracted prior to amplification.

Introduction

Urinary tract infections (UTIs) are bacterial infections of the urinary system and can be caused by various bacterial species, such as *E. coli* [1]. Identification of urinary tract microbial pathogens traditionally occurred through culturing of a presumptive positive sample. However, culturing of samples requires a considerable amount of time and can miss polymicrobial samples. The advancement of the genomic age has introduced numerous improvements to our identification of microbial pathogens including the addition of qPCR and next-generation sequencing (NGS) [2]. While NGS provides a wealth of information, the turnaround time associated with sequencing can be days to weeks, as with culture-based methods, and can cost considerably more than qPCR-based tests.

qPCR is gaining popularity within the research community as a reliable method for novel microbial identification assays. qPCR offers distinct advantages over NGS and cell culture including a reduction in cost and turnaround time. In addition to the above advantages, qPCR also allows for improved discrimination between pathogens and availability to query multiple targets in a single reaction through multiplexing assays. Traditionally, however, steps were needed to extract nucleic acid from the sample of interest, and this would slow the time from sample collection to results by 1–2 hours. IDT developed the PrimeTime One-Step 4x Broad-Range Master Mix to help researchers change their approach to crude sample preparation for a more streamlined qPCR workflow (Figure 1). This master mix allows researchers to skip the timely extraction step and go directly from sample collection to qPCR without loss in amplification efficiency. Furthermore, when taking typical reagent costs into account, we estimate that eliminating the extraction step will save researchers $1-2 per sample per extraction. Below we will explore direct amplification of urine samples and compare the data to amplification results from the same samples following traditional extraction.

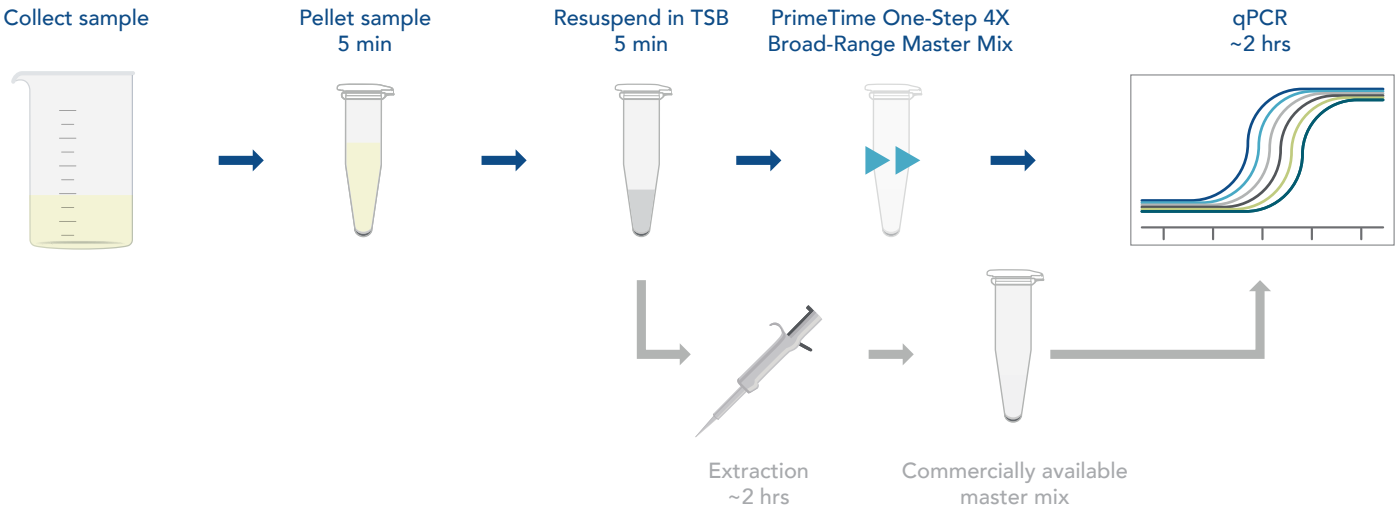

Figure 1. Overview of bacterial identification qPCR workflow using urine samples. Using the PrimeTime One-Step 4X Broad-Range Master Mix rather than a traditional qPCR master mix saves time by giving researchers the option to skip the extraction step.

Methods

Sample preparation—controls and human urine samples

For each bacterial target species, gBlocks™ Gene Fragments (IDT) synthetic controls were designed against a target amplicon with a five base spacer added to the 3′ and 5′ ends. The gBlocks Gene Fragments were used in a dilution series from 100,000 to 10 copies per reaction with data shown for 1000 copies per reaction. For biological controls, whole organism controls (ATCC or Zeptometrix) were used and bacterial suspensions were normalized to 0.5 McFarland standards.

For human urine samples, 1 mL of urine was concentrated by centrifugation, supernatant removal, and sample resuspension in 1 mL of Tryptic Soy Broth (TSB). Extractions were completed on a KingFisher™ instrument (Thermo Fisher Scientific) using 300 µL of the TSB resuspended sample.

Quantitative PCR

qPCR was performed using PrimeTime One-Step 4X Broad-Range Master Mix with enhancer solution (IDT), IDT custom DNA oligos for primers (300 nM final concentration), and either PrimeTime qPCR Probes or Affinity Plus™ qPCR Probes (150 nM final concentration) (IDT). For urine samples, 5 µL of either the extracted or unextracted samples were added to the reaction mix. Cycling conditions were 15 min. 50°C; 3 min. 95°C; 40 x (15 sec. 95°C; 1 min. 60°C).

Results

qPCR amplification of extracted urine samples

Typical research workflows for UTI-associated bacteria identification require the collection of urine and subsequent extraction of the resulting sample prior to qPCR amplification to purify DNA and remove qPCR inhibitors. To establish a baseline for amplification results using the PrimeTime One-Step 4X Broad-Range Master Mix with standard methods, data from extracted urine samples was collected by targeting eight different UTI-associated bacterial targets and compared to amplification data collected from gBlocks Gene Fragments controls. The data shows that qPCR testing of two extracted urine samples identified multiple UTI-associated bacteria but resulted in higher threshold cycle (C_t) values compared to reactions that included synthetic controls (Figure 2A). This motivated the pursuit of alternative approaches, such as extraction-free qPCR, for improving amplification from urine samples for bacterial identification while also expediting the research process.

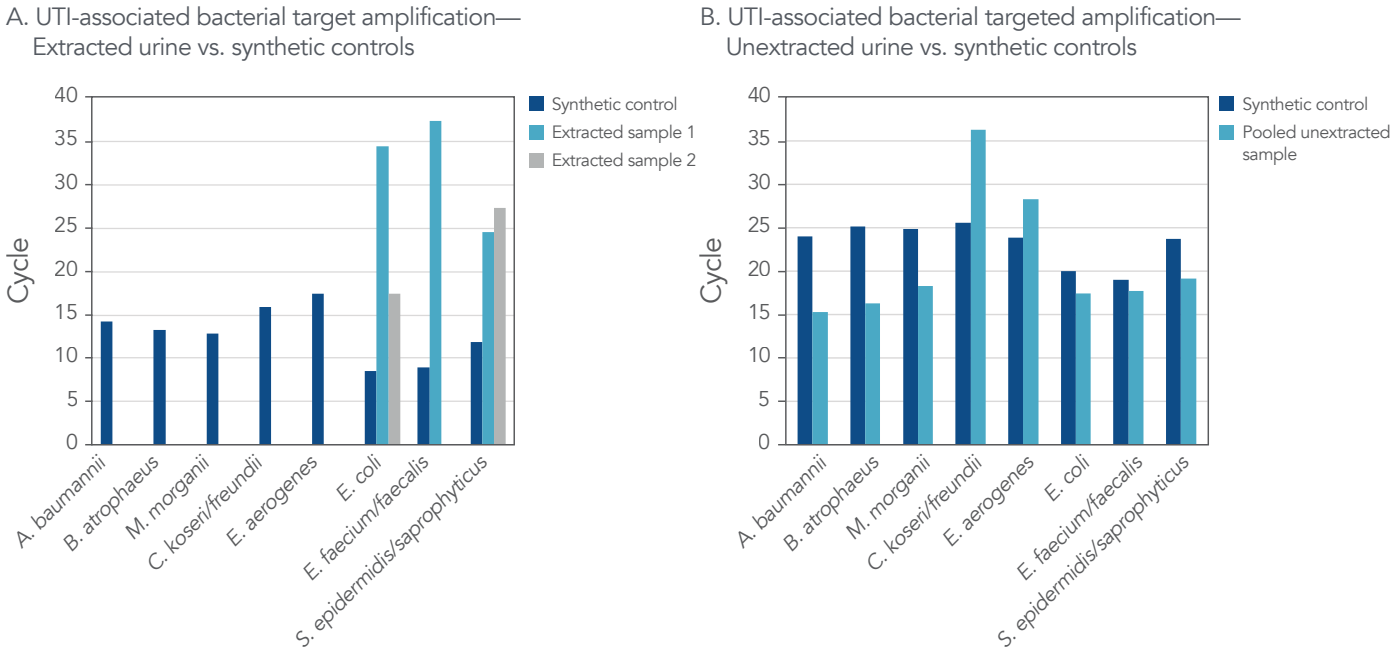

Figure 2. Amplification data for UTI-associated bacterial targets from extracted and unextracted research samples. (**A**) Two extracted urine samples positive for various UTI-associated bacteria were qPCR amplified and compared to qPCRs using gBlocks Gene Fragments synthetic controls as template for amplification (1000 copies per reaction). (**B**) Pooled biological samples positive for all 8 targeted UTI-associated bacterial species (*Acinobacter baumannii* [FAM], *Bacillus atrophaeus* [SUN], *Morganella morganii* [Cy5], *Citrobacter koseri/freundii* [FAM], *Enterobacter aerogenes* [SUN], *Escherichia coli* [Cy5], *Entercoccus faecium/faecalis* [FAM] and *Staphylococcus epidermidis/saprophyticus* [SUN]) were amplified in multiplex qPCRs without prior extraction and compared to qPCRs using gBlocks Gene Fragments synthetic controls (1000 copies per reaction). qPCR conditions for both experiments (**A-B**) were as described in the Methods section (n = 1).

Direct amplification of biological samples

Here we present data from a direct amplification qPCR workflow using extraction-free biological samples amplified with IDT's PrimeTime One-Step 4X Broad-Range Master Mix. Data was collected across eight different UTI-associated bacterial targets and amplification results from the unextracted urine samples were compared to reactions using synthetic gBlocks Gene Fragments controls (Figure 2B). The data shows that the extraction-free samples amplify as well or better than the gBlocks Gene Fragments controls for six of the eight bacterial targets tested. For the two targets (*C. koseri/freundii* and *E. aerogenes*) that did not amplify as well from biological samples, additional work is needed to determine if the increased C_t was the result of direct amplification or related to variations in sample input.

Conclusions

The results demonstrated here show that the use of IDT's PrimeTime One-Step 4X Broad-Range Master Mix is an effective solution for direct amplification from urine samples to identify targeted sequences from UTI-associated pathogens. The implementation of direct amplification results in faster progression from sample collection to data analysis and can reduce overall experimental costs by removing costly extraction steps.

References

1. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. **Urinary tract infections: epidemiology, mechanisms of infection and treatment options**. *Nat Rev Microbiol*. 2015;13(5):269-84.
2. Franco-Duarte R, Cernakova L, Snehal Kadam, *et al*. **Advances in Chemical and Biological Methods to Identify Microorganisms-From Past to Present**. *Microorganisms*. 2019;7(5):130.

Additional information and related protocols can be found at **www.idtdna.com/BroadRange**.

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For more information, go to: **www.idtdna.com/ContactUs**

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