Direct amplification of research samples associated with upper respiratory infections

Abstract

Biological extractions are essential for collection of nucleic acids and removal of PCR inhibitors prior to quantitative PCR (qPCR). In this document, we will introduce you to IDT's PrimeTime™ One-Step 4X Broad-Range Master Mix as an alternative to the costly and time-consuming extraction steps. Here, we show that the addition of the PrimeTime One-Step 4X Broad-Range Master Mix Amplification Enhancer solution (included with the Master Mix) produces better direct amplification of extraction-free saliva and nasal pharyngeal samples.

Introduction

Respiratory infections are bacterial or viral infections of the respiratory tract system. Microbial identification traditionally occurs through qPCR following extraction of DNA or RNA from an upper respiratory sample. Early on during the global SARS-CoV-2 pandemic, reagents like extraction kits became scarce and pushed the research community to look for alternatives.

qPCR is gaining popularity within the research community as the go-to method. 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 increased fidelity in discriminating between pathogens and availability to query multiple targets in a single reaction through multiplexing assays. Traditionally, steps were needed to extract nucleic acids from the sample of interest, and this would slow down the time from sample collection to results by 1–2 hours. The advent of IDT's PrimeTime One-Step 4x Broad-Range Master Mix is changing how researchers approach their experimental set-up. This Master Mix gives researchers the opportunity to skip the timely extraction step and go directly from sample collection to amplification without a loss in efficiency. A basic outline of the workflow is shown in Figure 1. Furthermore, eliminating the extraction step will save researchers $1–2 per sample per extraction. Below, we will explore direct amplification of cell culture samples and how the data compare to normalized controls.

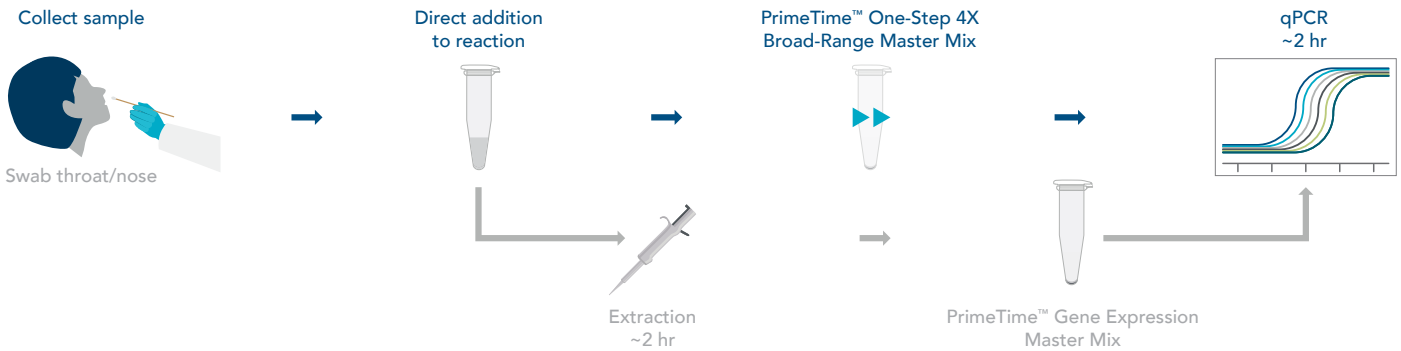

Figure 1. Basic workflow of viral testing of saliva and nasal pharyngeal samples. Utilizing the PrimeTime One-Step Broad-Range Master Mix gives researchers the opportunity to shorten the workflow by skipping the lengthy and costly extraction step.

Materials and Methods

Human samples

For human saliva or nasal pharyngeal (NP) samples, 2 µl of saliva or viral transport media were used per reaction for direct amplification or 5 µl of extracted sample.

Quantitative PCR

qPCR was performed using PrimeTime One-Step 4X Broad-Range Master Mix with enhancer solution (Integrated DNA Technologies) with custom DNA oligos for primers (Integrated DNA Technologies) and PrimeTime qPCR Probes (Integrated DNA Technologies) (Table 1). Cycling conditions for extracted samples were: 50°C for 15 min, 95°C for 3 min followed by 45 cycles at 95°C for 15 sec, then 55°C for 30 sec. Cycling conditions for direct amplification were: 50°C for 15 min, 95°C for 30 sec followed by 45 cycles at 95°C for 5 sec, then 55°C for 45 sec.

Table 1. Oligo sequence and concentrations used in this study.

Oligo name	Sequence	Concentration per reaction
InfA For1	CAAGACCAATCYTGTCACCTCTGAC	400 nM
InfA For2	CAAGACCAATYCTGTCACCTYTGAC	400 nM
InfA Rev1	GCATTYTGGACAAAVCGTCTACG	600 nM
InfA Rev2	GCATTTTGGATAAAGCGTCTACG	200 nM
InfB For	TCCTCAAYTCACTCTTCGAGCG	800 nM
InfB Rev	CGGTGCTCTTGACCAAATTGG	800 nM
SC2 For	CTGCAGATTTGGATGATTTCTCC	800 nM
SC2 Rev	CCTTGTGTGGTCTGCATGAGTTTAG	800 nM
RnaseP For	AGATTTGGACCTGCGAGCG	800 nM
RnaseP Rev	GAGCGGCTGTCTCCACAAGT	800 nM
InfA Pro	/56-FAM/TGCAGTCCT/ZEN/CGCTCACTGGGCACG/3IABkFQ/	200 nM
InfB Pro	/5YakYel/CCAATTCGA/ZEN/GCAGCTGAAACTGCGGTG/3IABkFQ/	200 nM
SC2 Pro	/5TexRd-XN/ATTGCAACA/TAO/ATCCATGAGCAGTGCTGACTC/3IAbRQSp/	200 nM
RnaseP Pro	/5Cy5/TTCTGACCT/TAO/GAAGGCTCTGCGCG/3IAbRQSp/	200 nM

Results

Efficient direct amplification of upper respiratory samples

Nasal pharyngeal collection of upper-respiratory samples is considered the preferred method for infectious disease research. To establish a baseline for direct amplification performance, NP samples were queried for Influenza A (Flu A), Influenza B (Flu B) or SARS-CoV-2 or the human *RNaseP* gene, as a control. Two separate reaction conditions were established with one condition using the Direct Amplification Enhancer and the other condition without the Enhancer (Figure 2A). While both conditions amplified for all four targets, the addition of the Direct Amplification Enhancer resulted in a more efficient amplification compared to the condition without the Enhancer (Figure 2A).

Next, saliva samples were collected, and the same experiment was performed with two separate conditions, one set receiving the Direct Amplification Enhancer and the other set without the Enhancer (Figure 2B). Unlike the NP swab samples, amplification was minimal in the sample set not receiving the Direct Amplification Enhancer. These results are not surprising as saliva is known to contain a higher level of PCR inhibitors than NP upper respiratory samples. These results demonstrate that the addition of the Direct Amplification Enhancer can neutralize PCR inhibitors and provide efficient direct amplification of saliva and NP swab samples.

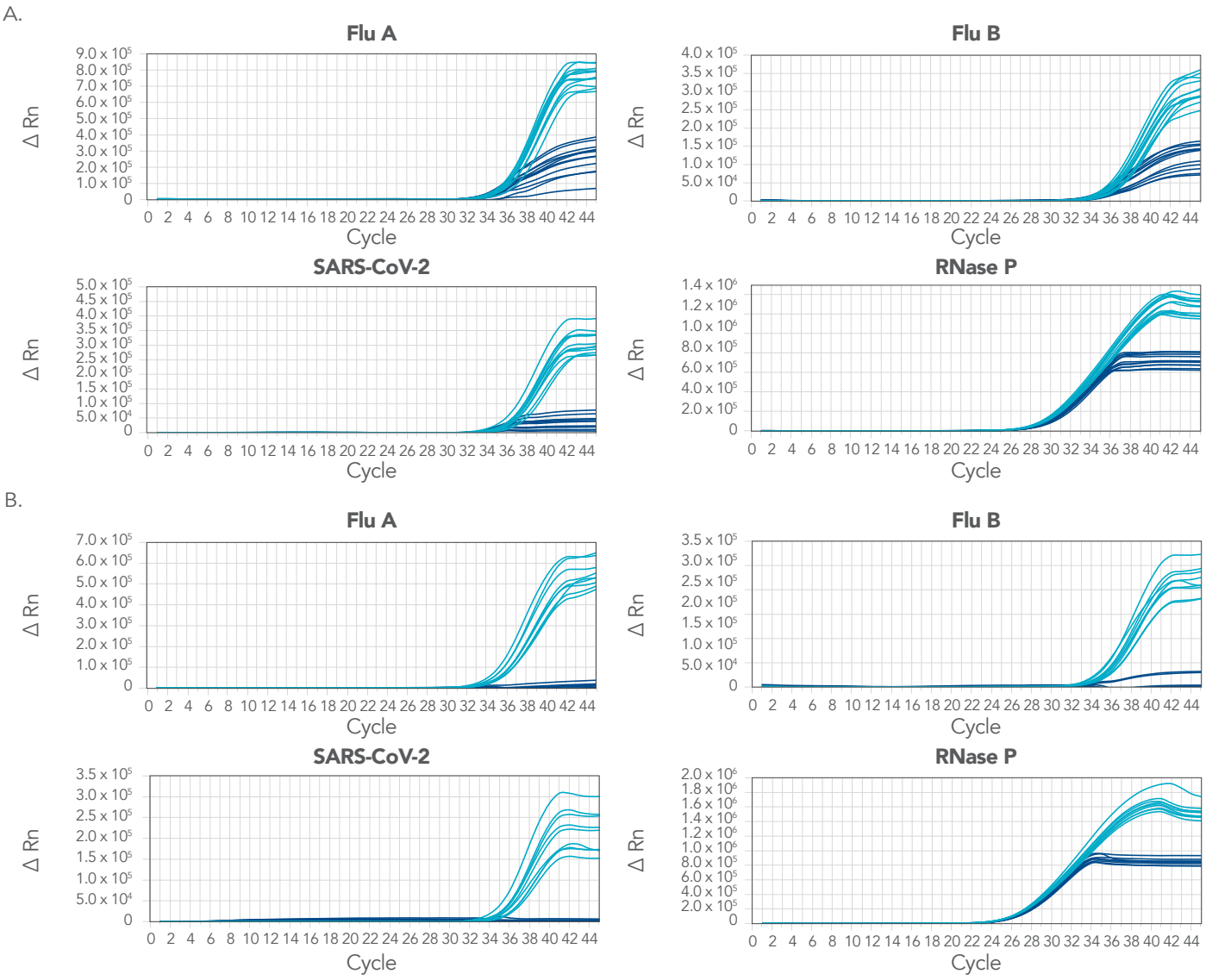

Figure 2. Amplification data for upper-respiratory viral targets from nasal pharyngeal and saliva collected samples. Amplification of three different viral targets using samples collected by (**A**) nasal pharyngeal (n = 12) (**B**) saliva (n = 9). PrimeTime One-Step 4X Broad-Range Master Mix was used with (light blue curves) or without (dark blue curves) the included Direct Amplification Enhancer.

Direct amplification outperforms extracted samples

Data from Figure 2 demonstrate that direct amplification of saliva and NP samples performs better when the Direct Amplification Enhancer is used with the PrimeTime One-Step 4X Broad-Range Master Mix, likely the result of inhibitors present in the samples. Next, we explored the addition of extraction on amplification performance of saliva and NP swabs. The two sample types were either added directly to the reaction or run through the KingFisher™ MagMAX™ (Thermo Fisher Scientific) extraction protocol prior to their addition to the PCR reaction. The direct amplification reactions also included the Direct Amplification Enhancer whereas the samples run through extraction did not. Interestingly, the saliva sample run through extraction did not perform nearly as well as the directly amplified saliva sample (Figure 3A). The data demonstrate equal amplification performance for the SARS-CoV-2 assay for both reaction conditions (Figure 3B). While the data in Figure 2 show the presence of inhibitors in the saliva samples, these results also demonstrate that some of the inhibitors present within saliva samples are carried through the extraction process into the PCR reaction. These results suggest that direct amplification of samples containing inhibitors can perform as well, or better, than samples run through traditional extraction protocols.

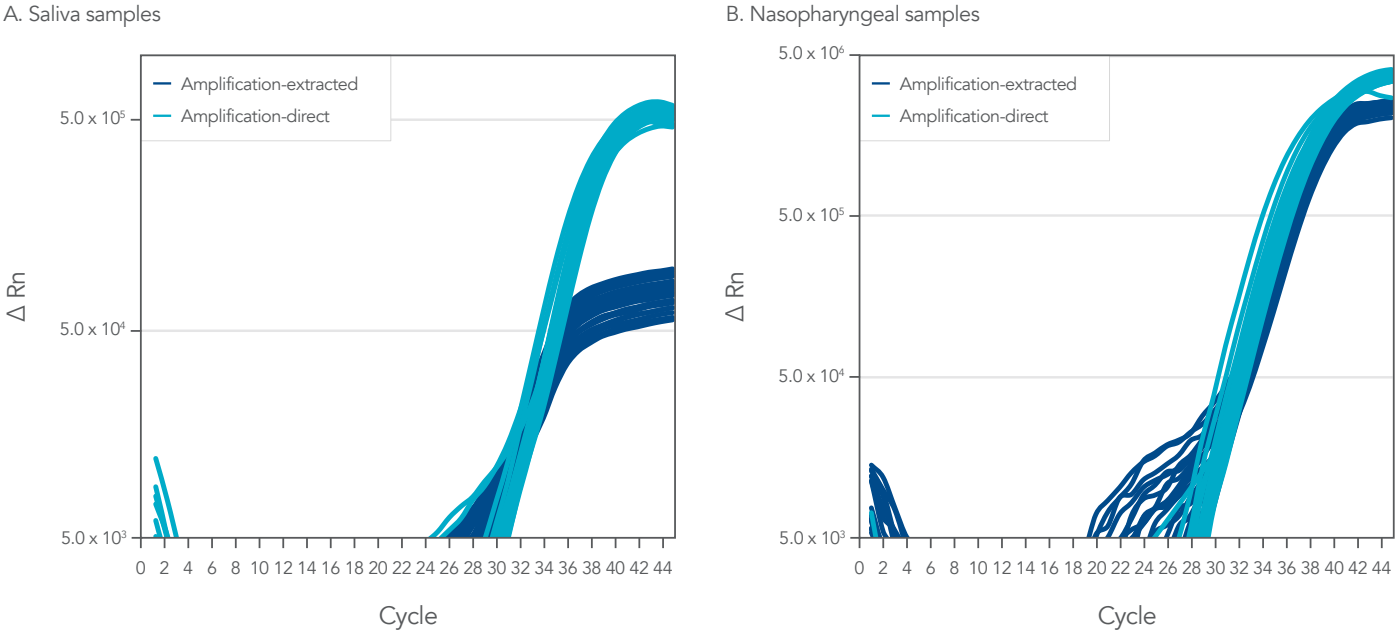

Figure 3. Amplification data for upper respiratory viral targets from saliva and nasal pharyngeal collected samples. Amplification of three different viral targets using samples collected by (**A**) saliva (*n* = 20) and (**B**) nasal pharyngeal (*n* = 20). PrimeTime One-Step 4X Broad-Range Master Mix was used with the included Direct Amplification Enhancer and no extraction (light blue curves) or without the included Direct Amplification Enhancer and extraction using the KingFisher MagMAX II RNA extraction system (dark blue curves).

Conclusion

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 of saliva and nasal pharyngeal research samples for identification of pathogens associated with upper respiratory infections. Direct amplification saves time and can reduce overall experimental costs by removing expensive extraction steps.

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