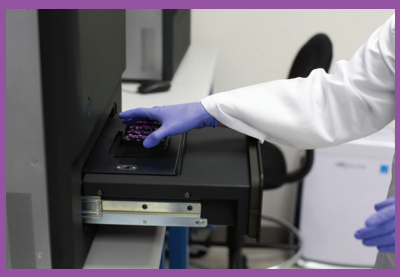
COMBINATI

1-Step RT-dPCR in under 80 Minutes on the Combinati Absolute Q

TRUE 1-STEP RT-DPCR

PREPARE
Reagent preparation is identical to qPCR

LOAD
dPCR reagents are loaded onto MAP16 plate and gaskets applied



START
Sample partitioning, reverse transcription, thermal cycling, and dPCR data aquisition
one instrument - 90 minutes

Background

Reverse Transcription PCR (RT-PCR) is an important tool that allows the assessment of nucleic acid targets that are present in the form of RNA. It has a wide range of applications including gene expression and detection of RNA viruses. During 1-step RT-PCR, reverse transcription of RNA to cDNA occurs in the same reaction vessel as the PCR, which is especially important for clinical applications as the reduced manual handling improves consistency and reduces time to result. Reverse transcription digital PCR (dPCR) further improves the technique by making quantification of extremely rare target material possible without the need for a comparative standard curve – thus enabling better overall consistency and lower limits of detection. Furthermore, recent data suggests dPCR outperforming qPCR in the detection of viral targets such as the widespread SARS-CoV-2 virus.[1]

The Combinati Absolute Q is a novel 4-color dPCR platform with a complete workflow identical to qPCR. This system overcomes many challenges presented by current dPCR workflows. For example, dPCR typically requires a minimum of 2 instruments to execute the thermal cycling and data collection steps separately. This increases both the time to answer and hands on time as the user is required to move the samples from one stopping point to the next. The Absolute Q's unique architecture allows it to handle reagent partitioning, reverse transcription, thermal cycling and data collection all on a single instrument and single consumable, enabling a true 1-step RT-dPCR workflow in under 80 minutes. In this technical note, we showcase 1-step RT-dPCR on the Absolute Q using the **|Q| SARS-CoV-2 Triplex Assay** using an RNA-based reference material.

Workflow and Materials

The **SARS-CoV-2 Triplex Assay** was designed using published CDC sequences as a single tube solution for SARS-CoV-2 identification and quantification with an integrated control assay for human gDNA. Using the Exact Diagnostics SARS-CoV-2 control material as input, we prepared the **SARS-CoV-2 Triplex Assay** according to

table 1. In addition to quantification of the RNA-based standard as a proof of concept for 1-step RT-dPCR, human genomic DNA alone and water controls were included.

Reagent	1 Reaction
Combinati 4X 1-step RT-dPCR MM	2.5 µL
COVID-19 10X Assay	1 µL
SARS-CoV-2 Standard *	1 µL
Water	5.5 µL

Table 1. 1-step RT-PCR Reaction mix formula.

*1µL of the control standard was loaded per reaction directly from stock. For negative control using human genomic DNA, 50 nanograms were loaded per reaction. Water was adjusted to accommodate the changes in sample volume.

Absolute Q Workflow

After preparing the dPCR mix, 10µL of the reaction mixture was loaded into the MAP16 plate followed by an overlay of 10µL of isolation buffer. The prepared MAP16 plate is then loaded on the Absolute Q. Table 2 details the thermal cycling protocol for RT-dPCR on the Absolute Q.

Temperature	Duration (M:SS)	Cycles
50°C	10:00	1
95°C	3:00	1
95°C	0:00	45
55°C	0:00	

Table 2. 1-step RT-PCR parameters on the Absolute Q

Quantification of SARS-CoV-2 Targets using reference materials

Unlike traditional RT-qPCR, RT-dPCR does not require a standard curve or reference sample to identify and quantify targets. The SARS-CoV-2 standard contains synthetic RNA targets from 5 genes of the novel coronavirus and human genomic DNA, which can be used to validate extraction methods. The Combinati SARS-CoV-2 Triplex assay targets the N1 and N2 gene sequences as well as the human RnaseP gene. As a demonstration of consistncy of one-step RT-dPCR, and using the control RNA as input, we performed the viral quantitation assay in duplicate across 4 separate instruments for a total of 8 replicates using this material.

We identified all three targets in the sample across 8 replicates and saw very few false positives within negative control reactions. We calculated the average concentration of viral and human targets and the associated standard deviation of the SARS-CoV-2 standard in copies per microliter - N1: 358.1 cp/µL (± 22.5), N2: 333.9 cp/µL (± 17.5) and RnaseP: 323.1 cp/µL (± 18.7). Fewer than one positive partition per dPCR reaction was identified on average across all replicate no template control reactions. For the Human Male Control reactions these values were: N1: 0.0 cp/µL (± 0.0), N2: 0.3 cp/µL (± 0.8). For water only, no template control reactions, these values were: N1: 0.4 cp/µL (± 0.9), N2: 0.1 cp/µL (± 0.4), RnaseP: 0.0 cp/µL (01770.0). Quantitation results were consistent across all four instruments (Figure 1).

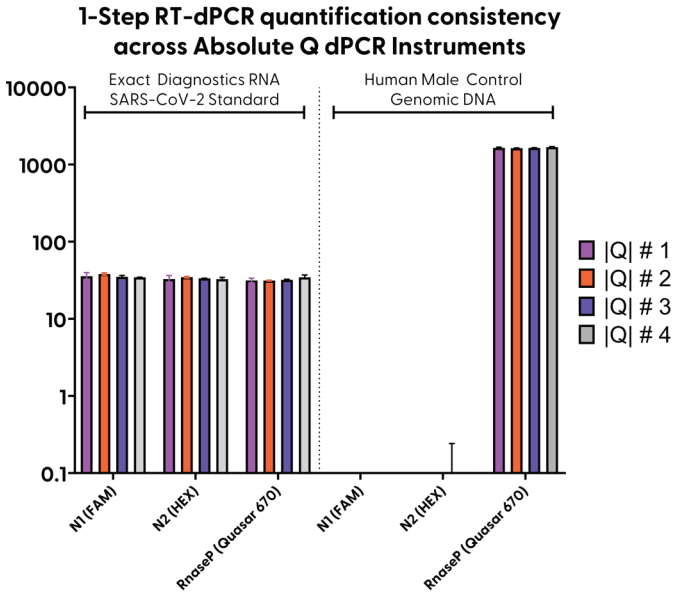


Figure 1. Cross instrument Absolute Q quantification consistency using 1-step RT-dPCR. Data shown are the results of the Combinati SARS-CoV-2 Triplex Probe Assay testing the RNA-based SARS-CoV-2 Standard Control ((Exact Diagnostics) and 50 nanograms of Human Male Control DNA (Promega) as a negative control for viral targets. Reactions were run in duplicate for each control material across four instruments for a total of eight replicates each.

Summary

The Absolute Q dPCR platform and its 80-minute 1-step RT-dPCR technology have broad implications for characterizing infectious diseases beyond COVID-19. The versatile platform can be adapted to a wide range of nucleic acid detection applications requiring absolute quantification. The Absolute Q simplifies dPCR with best-in-class data consistency, a short sample-to-answer time, and flexible multi-color multiplexing capabilities. Combinati aims to lower the barrier to bring dPCR into the lab to accelerate the response to global public health emergencies such as the COVID-19 pandemic.

References:

Dong L, Zhou J, Niu C, et al. Highly accurate and sensitive diagnostic detection of SARS-CoV-2 by digital PCR. medRxiv. March 2020:2020.03.14.20036129. doi:10.1101/2020.03.14.20036129

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