SARS-CoV-2 Reference Material Quantification on the Absolute Q dPCR Platform

Highlights

- **The Absolute Q provides best-in-class nucleic acid analysis with a complete 90 minute walk-away dPCR workflow.**
- **Linear dynamic range verified down to 10 SARS-CoV-2 copies per reaction volume**
- **Absolute quantification of reference material will ensure consistent assay performance and disease reporting**
- **Accurate dPCR quantification of SARS-CoV-2 targets will benefit the global pandemic response.**

Introduction

The rapid outbreak of COVID-19 originating in Wuhan, China has mobilized unprecedented response to the pandemic across the globe. Numerous diagnostic tests have been deployed to aid in control of disease spread. Positive control materials are required for assay development and to assess overall consistency. To ensure that COVID-19 tests have consistent limits of detection, accurate quantitative measurement of these control materials is critical.

To address the need for a reliable method of accurate and precise measurements of SARS-CoV-2 samples, we leverage the Combinati Absolute Q Digital PCR (dPCR) platform, to quantify commercially available SARS-CoV-2 reference control material. The Absolute Q simplifies dPCR to a single-step, under 90min workflow with best-in-class partitioning and data consistency.

Workflow and Methods

COVID-19 Assays and Reference Materials

For this study, the IDT 2019-nCoV kit were used for all reactions on the Combinati Absolute Q platform. Per the CDC's guidelines, only the N1 and N2 assays were used. Both DNA and RNA reference materials were evaluated in this study. The Combinati 2X PCR MaestroMix was used for DNA reference material and the UltraPlex™ 1-step ToughMix® (QuantaBio) for RNA reference material. All PCR mixes were prepared to a final concentration of 500nM for each primer and 125nM viral-target specific probe.

All controls contain synthetic sequences of gene targets relevant to the SARS-CoV-2 virus. The reference materials evaluated were either ready-to-use nucleic acids, or required pre-processing before use. The DNA reference obtained from IDT (2019-nCoV_N_Positive Control), and RNA reference obtained from Exact Diagnostics (SARS-CoV-2 Standard) were both ready to use. The SeraCare AccuPlex™ Coronavirus SARS-CoV-2 Reference Material is comprised of RNA encapsulated within a mammalian cell, requiring an extraction step prior to use.

Temperature	Duration	Cycles
25°C	30 minutes	1*
50°C	15 minutes	1*
95°C	2 minutes	1
95°C	0 seconds	55 cycles
55°C	0 seconds	

*Included only for RNA templates requiring reverse transcription

Table 1. Thermal cycling parameters for SARS-CoV-2 assays on the Absolute Q

For general quantification of the ready-to-use reference material, 1µL of volume was used as input for the PCR reaction. To quantify the SeraCare reference material, the maximum sample volume of 6.75µL was loaded into each assay after RNA extraction. A dynamic range and consistency evaluation across multiple Absolute Q instruments was conducted to demonstrate assay performance and instrument consistency.

Absolute Q Instrument Parameters

All prepared PCR mixes were loaded directly onto the Combinati **M**icrofluidic **A**rray **P**artitioning (MAP16) consumable and subsequently placed onto the Absolute Q dPCR instrument. The CDC's guidelines for thermal cycling parameters for qPCR were optimized for the Absolute Q which enables shortened PCR cycle dwell times - as noted in Table 1. Additional steps were included for the RNA control material which required reverse transcription as an initial step. Digitization, thermal cycling and dPCR data collection are integrated into the Absolute Q's one-step walkaway workflow which was completed in under 90 minutes.

Results

Ready-to-use reference material quantification

The 2019-nCoV Reference Material (DNA) and SARS-CoV-2 Standard (RNA) were both quantified using the N1 and N2 assays with 1µL of sample input using the Absolute Q dPCR system. Across all replicates, the average concentrations for each viral target were consistent between reference materials (Figure 1).

Dynamic range evaluation

A dynamic range series, targeting 1000, 100 and 10 copies per reaction was generated alongside a no template control (NTC)

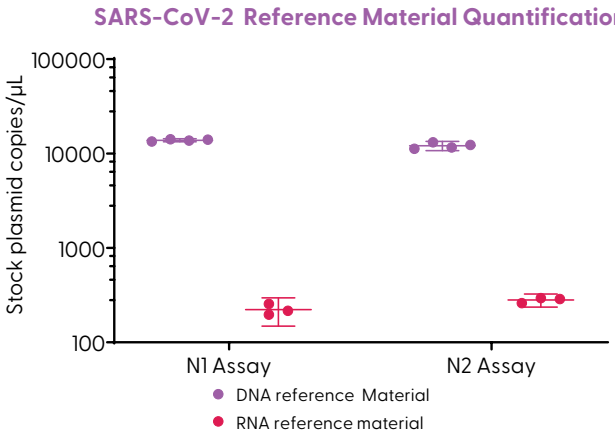

Figure 1. Absolute Q dPCR results. 2019_nCov_N_Positive Control: 13,815 cp/µL ±381, N=4 and 12,706 ±845 cp/µ, N=4 for N1 and N2 targets respectively. SARS-CoV-2 Standard: 222 cp/µL ±30, N=3 and 281 cp/µL ±18, N=3 for N1 and N2 targets respectively.

using the DNA reference material. For each point, two replicates each of the N1 and N2 assays were prepared. The complete dynamic range set was repeated across three separate Absolute Q instruments for a total of 6 replicates per point (Figure 2).

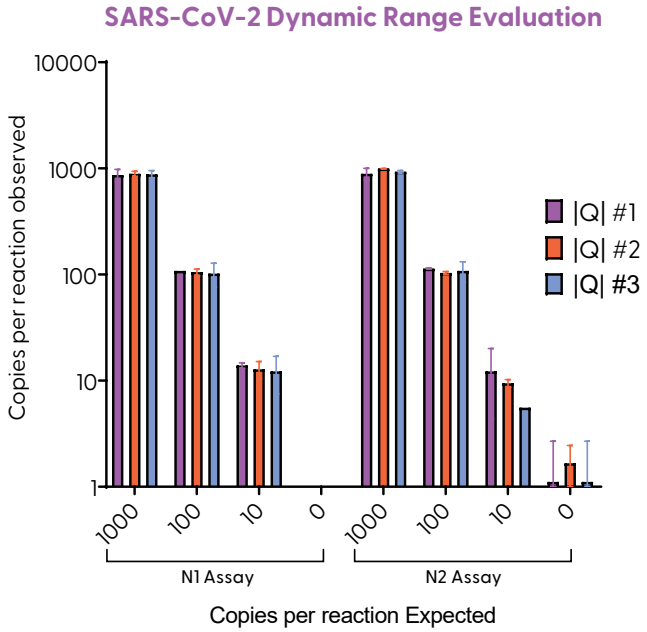

Figure 2. Multi-instrument consistency data for the two assays across three ten-fold dilutions of control reference material.

Extraction efficiency reference material quantification

Reference material which requires pre-processing enables assessment of extraction efficiency and other up-front processes involved in patient sample testing. We extracted RNA from the SeraCare reference material and added the maximum sample input into each of the CDC recommended assays. The concentration of SARS-CoV-2 material post extraction of 200µL and elution in 50µL was 7.9 cp/µL and 9.7 cp/µL for the N1 and N2 assays respectively.

Summary

The Combinati Absolute Q provides a walkaway digital PCR workflow. Considering all steps including reverse transcription are completed in under 90 minutes, the Absolute Q provides the fastest time-to answer digital PCR workflow for precise quantification of viral reference material.