ABSTRACT

Reliable assays are a necessity for timely processing of samples that must be tested for SARS-CoV-2. To improve the quality and reliability of viral detection by the reverse transcription quantitative PCR (RT-qPCR) method, it is important to mitigate potential delays along the workflow. The Thermo Scientific™ Acclaro™ Sample Intelligence technology built in to the Thermo Scientific™ NanoDrop™ One Microvolume UV-Vis Spectrophotometer offers a set of tools that help provide accurate nucleic acid concentrations, identify many contaminants, and prevent failed experiments.

INTRODUCTION

Testing considerations for SARS-CoV-2 samples

In early 2020, the World Health Organization published the genetic sequence for SARS-CoV-2, the virus that causes the COVID-19 disease. The widespread spread was classified as a pandemic on 11 March 2020. As of 3 August 2020, over 18 million individuals have tested positive for the virus around the world. Testing patients for the virus has been the primary focus for government organizations, hospitals, and private laboratories around the world. The Centers for Disease Control and Prevention describes several assays that can be used for the detection of the virus. There are multiple SARS-CoV-2 detection workflows applying RT-qPCR, antibody assays, electron microscopy, and more. The CDC guidelines do not mention nucleic acid quantification after RNA extraction or purification. For research use only. Not for diagnostic use.

ABSTRACT

Reliable RT-qPCR assays demand careful experimental design, extensive QC, and transparent data analysis. In 2009, leaders within the qPCR field published the MIQE guidelines to improve the quality and reliability of experiments. An RT-qPCR assay includes two enzymatic steps. In the first step, RNA is converted to cDNA using reverse transcriptase. Normalizing the amount of RNA going into the reaction helps minimize variability in cDNA production, which can be crucial since RT reaction efficiencies can change with different RNA concentrations. In the second step, gene specific primers are used in combination with cDNA template to determine the relative expression compared to various reference genes. Reference genes are an important part of this reaction because they are used to normalize small differences in cDNA input. If there are large differences in cDNA amount, normalization based on reference genes can become problematic. The MIQE guidelines require input RNA quantity and purity to be published.

Using NanoDrop One Spectrophotometers for qPCR workflows

The NanoDrop One Microvolume UV-Vis Spectrophotometer is trusted by scientists around the world for nucleic acid quantification of small volumes of sample. These instruments are built with Acclaro Sample Intelligence technology, which identifies the presence of many common contaminants in samples and calculates an approximate concentration of the contaminant. Common molecules found in nucleic acid extraction kits can overwhelm analytes of interest. To illustrate this, we extracted RNA from mouse liver and spiked it with a GuSCN contaminant (Figure 3). We used absorbance at 260 nm to determine the analyte concentration and set-up qPCR based on the uncorrected value.

Figure 3. The effect of guanidine thiocyanate on nucleic acid spectra. The absorbance at 260 nm is unaffected by the presence of GuSCN, but the A260/A280 ratio may be impacted.

Because guanidine salt contamination can inhibit qPCR by denaturing polymers, its presence could falsely elevate the cycle threshold (Ct) count. We measured Ct, noting that it increased with contaminated samples and indicated that the reaction did not go as planned (Figure 4). We attribute this to the presence of guanidine salts that inhibit polymers and block the reaction. Acclaro software built into the NanoDrop One can detect GuSCN in RNA samples and has features that can help prevent failed qPCR experiments (Figure 5 and Table 1).

Figure 5. Acclaro technology contamination alerts. Acclaro technology A) flags an alert for a contaminant present in a dsDNA sample with an icon (A) and B) offers additional details about the protein contaminant in the sample that contributes to A260 that infatuates dsDNA concentration. Acclaro provides uncorrected values and corrected values, where the protein contribution is subtracted from the original value to provide the corrected concentration.

Table 1. Contaminant range detectable by Acclaro technology.

<table>
<thead>
<tr>
<th>Contaminants detected</th>
<th>protein</th>
<th>protein</th>
<th>phenol</th>
<th>phenol</th>
<th>guanidine HCl</th>
<th>guanidine SCN</th>
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</thead>
<tbody>
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<td>dsDNA concentration</td>
<td>0.5A-2.5A at 260 nm</td>
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RESULTS

Several CDC-approved nucleic acid extraction kits use guanidine thiocyanate (GuSCN) and guanidine HCl as part of their chemistries. If GuSCN contamination is present in a sample, it will not drastically change the reported absorbance value at 260 nm or the calculated nucleic acid concentration. However, it can denature qPCR polymers. To illustrate this, we extracted RNA from mouse liver and spiked it with a GuSCN contaminant (Figure 3). We used absorbance at 260 nm to determine the analytic concentration and set-up qPCR based on the uncorrected value.

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CONCLUSIONS

The Acclaro Sample Intelligence technology in each NanoDrop One spectrophotometer helps improve confidence in sample assessment by identifying many common contaminants that could affect downstream RT-qPCR analysis. The algorithms provide corrected DNA or RNA concentration for each sample in under 8 seconds and calculate accurate A200:A280 and A260:A230 purity ratios. Having the ability to reduce costly delays from troubleshooting failed experiments by better understanding the original sample is a strongly desirable feature in time-sensitive assays.

TRADEMARKS, LICENSING & INTENDED USE

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