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Qualification of nucleic acid samples for infectious disease research workflows

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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 help prevent failed experiments.

Thermo Scientific NanoDrop One/One^{C}
Microvolume UV-Vis Spectrophotometer

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 disease spread and 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 including RT-qPCR, antibody assays, electron microscopy, and more[1] (Figures 1 and 2). The Applied Biosystems™ TaqPath™ COVID-19 Combo Kit and several qPCR instruments were granted emergency use authorization by the U.S. Food and Drug Administration in Q2 2020[4].

Figure 1. Multiple SARS-CoV-2 detection workflows

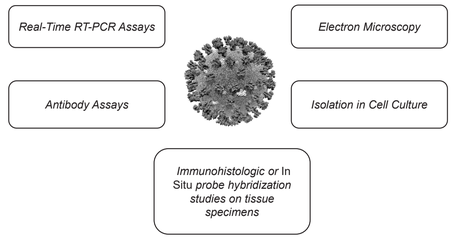
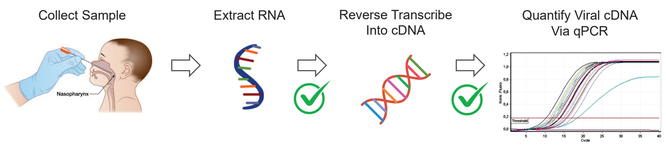


Figure 2. One SARS-CoV-2 workflow is a Real-Time RT-qPCR Assay. This figure is adapted from the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel[2]. Note that the CDC guidelines do not mention nucleic acid quantification after RNA extraction or before qPCR amplification. The MIQE guidelines for minimum information for publication of quantitative real-time PCR experiments[3] do define nucleic acid quantity and A260/A280 purity as useful information for qPCR publication.



Importance of nucleic acid quantification

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. During 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 are reported in publication.

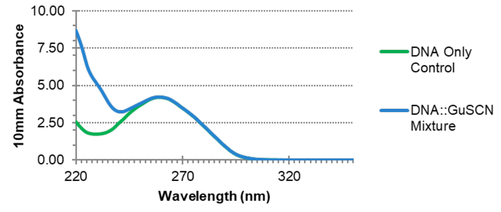
Using NanoDrop One Spectrophotometers for qPCR workflows

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 overestimate analyte concentrations or denature qPCR polymerases. In either case, the qPCR experiment can fail. The Acclaro software provides corrected nucleic acid concentrations and identifies many common contaminants, to help minimize time spent troubleshooting and managing failed experiments, which are especially critical for mitigating issues under pandemic timelines.

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 polymerases. 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/A230 ratio may be impacted.



Because guanidine salt contamination can inhibit qPCR by denaturing polymerases, its presence could falsely elevate the cycle threshold (Ct) count. We measured Ct, noting that it increased with contaminant concentration and indicating that the reaction did not go as planned (Figure 4). We attribute this to the presence of guanidine salts that inhibit polymerases 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 4. Mouse Liver RNA spiked with GuSCN.

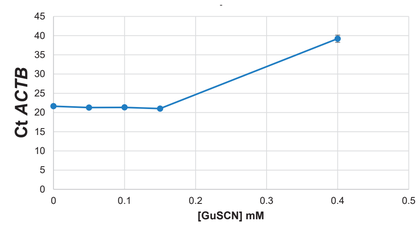
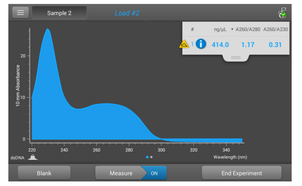


Figure 5. Acclaro technology contamination alerts. Acclaro technology A) flags an alert for a contaminant present in a dsDNA sample with an icon (⚠) and B) offers additional details about the protein contaminant in the sample that contributes to A260 that inflates dsDNA concentration. Acclaro provides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

A



B

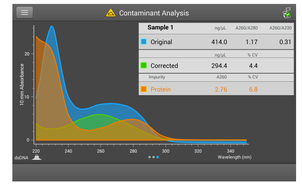


Table 1. Contaminant range detectable by Acclaro technology.

	dsDNA	RNA
Contaminants detected	• protein • phenol • guanidine HCl	• protein • phenol • guanidine SCN
Sample concentration range	0.5A-62.5A at 260 nm	0.5A-62.5A at 260 nm

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 A260/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.

REFERENCES AND NOTES

1. https://www.cdc.gov/sars/guidance/f-lab/assays.html
2. https://www.fda.gov/media/134922/download
3. https://www.ncbi.nlm.nih.gov/pubmed/19246619
4. **For Emergency Use Authorization (EUA) only. For prescription use only. For *in vitro* diagnostic use.**

TRADEMARKS, LICENSING & INTENDED USE

NanoDrop instruments are For Research Use Only. Not for use in diagnostic procedures. Applied Biosystems™ TaqPath™ COVID-19 Combo Kit and several qPCR instruments were granted Emergency Use Authorization by the U.S. Food and Drug Administration in April 2020.

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