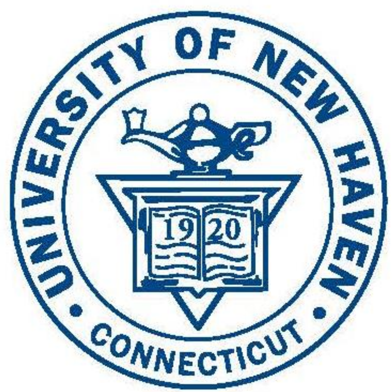

University of New Haven

A Comparison of the Quantitative Efficiencies of the ABI 7500 SDS (Real-Time PCR) versus NanoDrop One Instrumentation

By Raeghan Steelberg, David San Pietro PhD

Introduction

Accurate quantitation of DNA is important in forensic science. The Applied Biosystems 7500 Sequence Detection System (SDS) Real-Time PCR and Thermo Scientific NanoDrop One Microvolume UV-Vis Spectrophotometer (ND) are two instruments that quantitate DNA. Real-Time PCR (RT) is widely accepted as the method of quantitating DNA in forensics. The ND uses UV spectrophotometry to determine the concentration of DNA in a sample. The ND is more cost efficient, easier to use, and quicker than the SDS. This study focused on determining the precision of the ND instrument by comparing the results of the RT method to the UV spectrophotometry method.

Materials and Methods

Samples: DNA was extracted from buccal swabs of two donors with informed consent following Institutional Review Board approval. The QIAamp DNA Mini Kit and the manufacturer's spin protocol were used for the extraction. A total of 6 samples were extracted (1, 2, A, B, C, D) in 3 series with an extraction negative in each series.

Dilution Analysis: Six dilution series were prepared and analyzed according to Figure 1. Promega's Plexor HY System and its manufacturer recommended reactant quantities were used for the SDS measurements. Undiluted samples 1, 2, B, and C were also measured using the ND and the SDS. Statistics t-tests and the Plexor HY Analysis software were utilized for analysis of dilution series 3, 4, 5 and 6.

Thermo Scientific NanoDrop One Microvolume UV-Vis Spectrophotometer on left and Applied Biosystems 7500 Sequence Detection System on right.

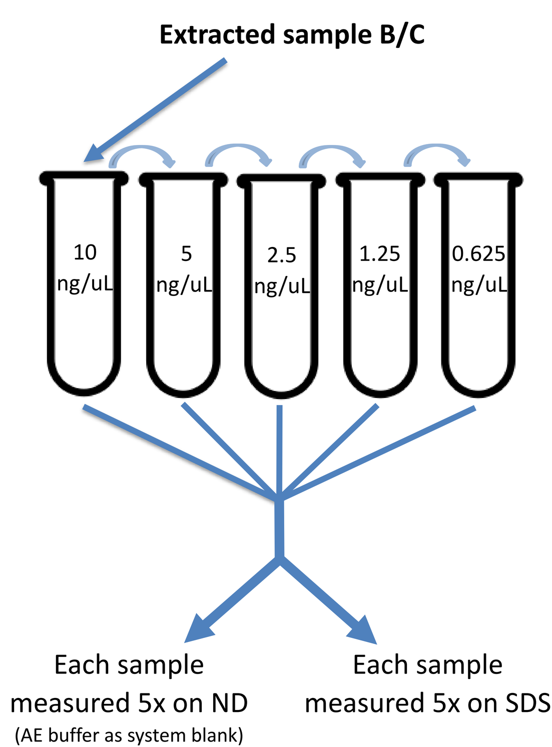

Figure 1 Method for one dilution series.

Results and Discussion

NanoDrop Precision: When the standard deviation values of the measurements made using the ND were compared for each sample concentration, the variation was consistent across each of the different concentrations. The average standard deviation values across all of the dilution series ranged from 0.25 ng to 0.37 ng. Although the standard deviation values remained about the same. The lower the concentration of the sample, the higher the amount of variation as compared to the concentration of the sample.

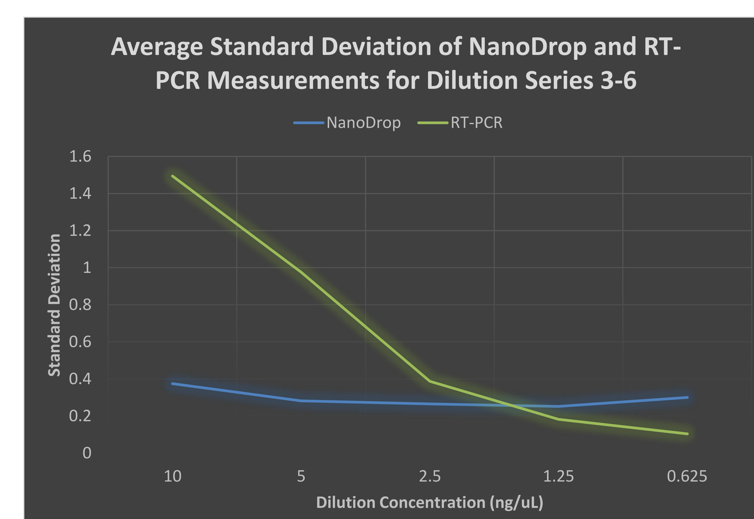

Figure 2 Average standard deviation for all measurements made on both measurements of the four dilution series.

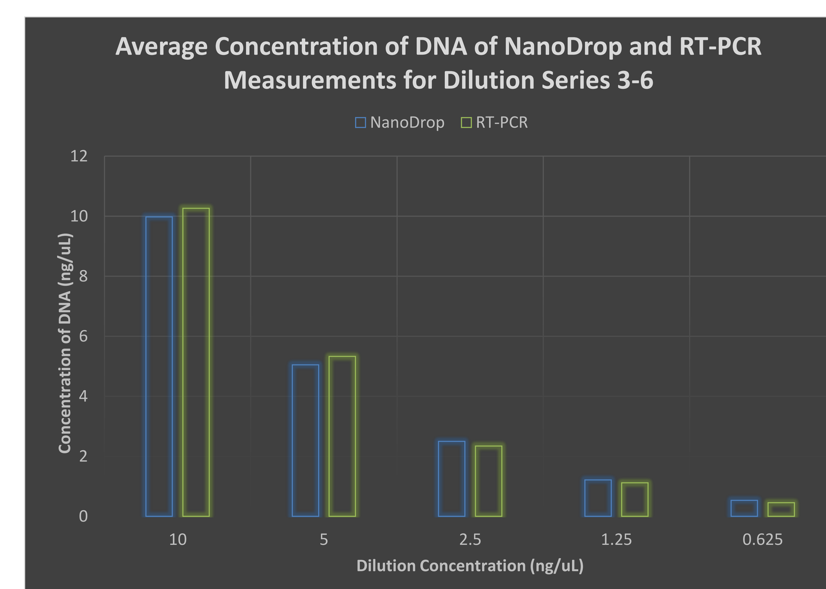

Figure 3 Average concentration of DNA for all measurements made on both instruments of the four dilution series.

Instrument Comparison: There were significant differences seen in the precision of the data from the two instruments. The dilution series measured with RT showed a decrease in the variation (standard deviation) as the concentration of DNA decreased. Contrastingly, the same dilution series measured on the ND did not show as much variation in the standard deviation as the concentration of DNA decreased. The amount of variation appeared to hold constant across all concentrations on the ND.
When comparing the average concentration of DNA from the RT and the ND of the same dilution series, t-testing showed that 75% of the time there was only significant variation ($p < 0.05$) at concentrations of 2.5 and 1.25 ng/uL between the two instruments. This can be explained by the very high standard deviation associated with the larger concentrations with the RT. The larger the range of values across all sample concentrations and their replicates, the more likely that the ND measurements would not appear to vary significantly compared to the RT measurements. It is likely that there was no significant variation at 0.625 ng/uL between the two instruments because the high standard deviation shown by the ND readings sufficiently overlapped with those of the RT. Therefore, the larger the range of variation across the dilution series as measured by the ND, the increase in likeliness that the RT values would not appear to vary significantly when compared to ND values. Undiluted neat aliquots of samples 1, 2, B and C were measured on both instruments and the concentrations of DNA found varied at the most by 16.5%.

Conclusions

Since low level amounts of DNA are common in forensic applications due to the nature of forensic evidence, the SDS is a better instrument for this purpose. RT is more precise at lower concentrations than the ND and as a result will lead to more optimal use of both the amplification and capillary electrophoresis (CE) processes, resulting in a quality DNA profile. The lower precision seen by the ND at lower levels of DNA can lead to the possible overloading/underloading of the amplification and CE steps in the DNA process. It is also common to find very limited amounts of sample in forensic casework evidence, so confidence in the DNA quantitation method is very important in optimizing the chances of getting viable results. Exemplar samples with larger amounts of DNA, like samples 1, 2, B, and C, could be measured using either quantitation method since the percent difference between the readings from both instruments was minimal.

Future Work

- Compare the DNA profiles of the samples using calculations according to ND and SDS measurements for the reactant quantities.
- Examine the accuracy of the ND and SDS by comparing to another "correct" method of DNA quantitation.
- Run more samples to better understand the relationship between the measurements of the ND and SDS and develop additional confidence in the conclusions made from this study.

References

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Acknowledgements

A special thanks to the University of New Haven SURF program for organizing the funding for this project. Also, thank you to Sandra Hartman-Neumann, the lab manager of the Forensic Science Department for her help in making everything run smoothly.