Internal Validation of a Robotic Liquid Handler for Real-time PCR Set Up

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Abstract

In an effort to reduce backlogs and increase throughput the Austin Police Department (APD) DNA Unit has automated many steps in analysis. The newest automation is a robotic liquid handler designed for real time PCR (rtPCR) set up. An internal validation was performed to ensure the reliability and precision of the robot. This validation study included evaluation of precision, contamination, comparison to manual methods, and mock case work. Standards prepared by the robot resulted in an average Ct standard deviation of 0.196 and an average slope of -3.26, showed no signs of contamination, and was shown to perform similar to validated manual methods.

Introduction

- Done according to American Society of Crime Laboratory Directors-Laboratory Accreditation Board (ASCLAD-LAB) accreditation requirements
- Studies done examining precision, contamination
- A comparison done between the robot and manual setup

Materials and Methods

- Samples were extracted with Qiagen's QiAmp™ investigator kit on the Qiacube™ extraction robot
- rtPCR quantification was done with Applied Biosystems Quantifiler™ on 7000™ and 7500™ Real Time PCR Systems
- Standard serial dilution prepared as seen in Table 1 with TE and 9947A
- Samples reactions were prepared according to the labs SOP
- Three precisions runs were done two with standards and one run with several aliquots of the same blood sample
- Blanks were used for the contamination study alternating by rows and columns
- Standards were prepared manually by the technical leader

Table 1: Display of the standard set and concentrations used to calculate the linear equation used for rtPCR quantification analysis.

Standard	Concentration (ng/µl)
Std 1	50.00
Std 2	16.70
Std 3	5.560
Std 4	1.850
Std 5	0.620
Std 6	0.210
Std 7	0.068
Std 8	0.023

Results

Precision

- Linear equations calculated and graphed for each set of standards (Figure 1)
- Quantitation values for the single sample aliquots were graphed (Figure 2)

Contamination

- No quantitation values were obtained for any reagent blank or negative control

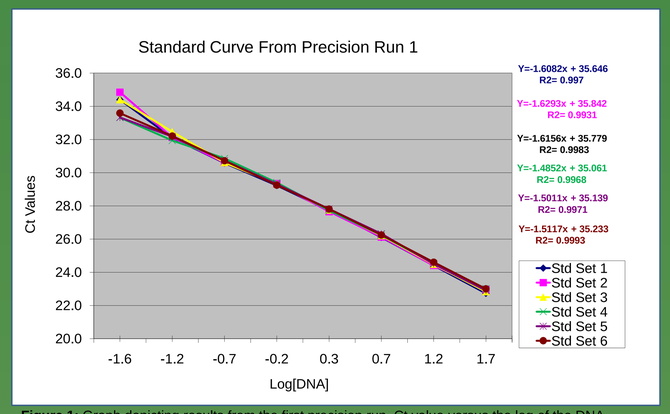

Figure 1: Graph depicting results from the first precision run. Ct value versus the log of the DNA concentration from each standard set were graphed. The linear equation for each standard set were calculated as shown above the legend.

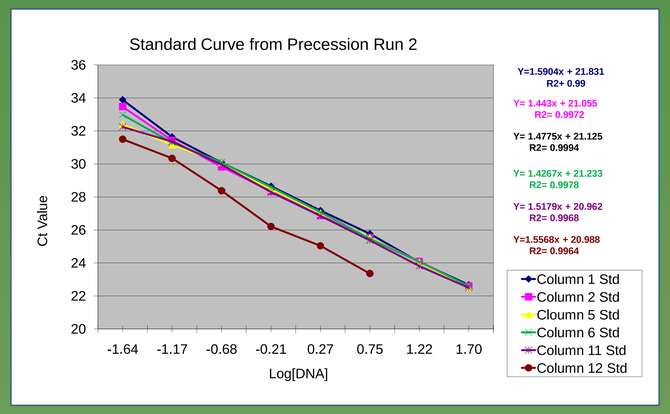

Figure 2: Graph depicting results from the second precision run. Ct value versus the log of the DNA concentration from each standard set were graphed. The linear equation for each standard set were calculated as shown above the legend. Note that Column 12 standard is much off of norm due do not mixing standards before set up.

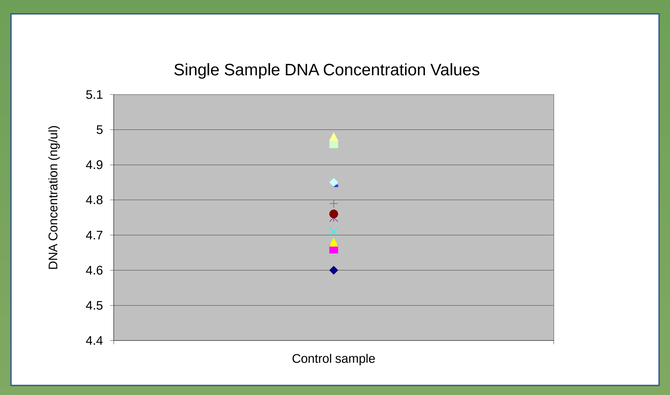

Figure 3: A cluster graph showing the different DNA concentration values from the same blood sample aliquoted with the robotic liquid handler.

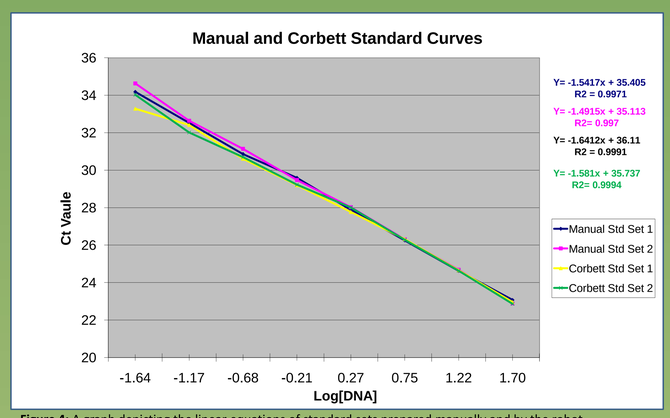

Figure 4: A graph depicting the linear equations of standard sets prepared manually and by the robot. Graph shows the comparison between the two methods of standard preparation.

Manual comparison

- Linear equations for each set of standards were calculated and graphed (Figure 4).

Mock Case Work

- Mock sexual assault samples quanted
- Resulting concentrations seen in Table 2

Table 2: Results from the mock case work quantization are bellow. The DNA concentration values are reasonable results for a sexual assault case.

Sample	Concentration (ng/µl)
3.1SF	1.80
3.1EC	0.898
4.1	0.494
1.1	0.354
2.1	0.585

Discussion

- Observed wider Ct range for the lowest DNA concentration (Std 8) is an expected result range for lower DNA concentrations
- Standard curve created from the last set of standards was a likely result of non-homogenized standards
- Standard curve slopes 95% confidence interval of -3.56 to -2.96, includes target -3.33
- The robot appears to result in no contamination given that no contamination was observed
- Robot prepared standards are consistent with or better then manually prepared standards

Conclusion

- Validation sufficiently demonstrated the liquid handlers accuracy and consistency
- A pre-mix step must be added prior to aliquoting standards for proper standard curve
- Robot shown to be precise, free of contamination and similar to current manual methods

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Acknowledgements

This project was supported by Award No. 2005-MU-BX-K020 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication/exhibition are those of the author(s) and do not necessarily reflect the views of the Department of Justice.