Comparative Evaluation of Three Commercial Quantitative PCR kits with Extremely Inhibited and Degraded Samples

Amy Sorensen, MS; Kyleen Elwick, BA; David Gangitano, PhD; Sheree Hughes-Stamm, PhD

Department of Forensic Science, Sam Houston State University, 1003 Bowers Blvd., Huntsville, TX 77340

INTRODUCTION

Forensic STR typing is currently the gold standard in the forensic DNA community. However, casework samples are often degraded, in low amounts and/or inhibited, which may complicate successful DNA typing. Severe inhibition can be mistaken for low template or severe degradation in a sample, or vice versa[1]. Therefore tools that better predict any DNA degradation and/or presence of PCR inhibitors in a sample prior to STR amplification could benefit laboratories by reducing the time for analysis and use of resources.

Various commercial quantitative PCR (qPCR) kits that simultaneously provide the amount of human and male DNA whilst also predicting the level of DNA degradation (via a degradation index; DI) and presence of PCR inhibitors in a sample are available to forensic DNA laboratories. These qPCR kits include a small and large human autosomal target in order to calculate the DI, in addition to a non-human target to determine the level of inhibition by comparing the internal PCR control (IPC) cycle threshold (C_T) of the sample to the C_T of the controls[2]:

a) Degradation Index :

$$\frac{\text{[DNA] of small target}}{\text{[DNA] of large target}}$$

b) Delta C_T :

$$\text{Sample IPC target } C_T - \text{Average of controls' IPC target } C_T$$

This study compared the performance of three commercial quantitative kits: 1) Quantifiler® Trio DNA Quantification Kit (Life Technologies), 2) PowerQuant® System (Promega), and 3) InnoQuant™ HY (InnoGenomics Technologies) with extremely inhibited samples and various degraded and damaged samples. Degraded samples include bone, decomposed, and formalin-damaged tissues and inhibited samples consisted of standard male DNA samples spiked with various concentrations of five inhibitors (hematin, calcium, humic acid, melanin, and salt in aqueous solution). The DNA concentrations, DI, and the delta C_T of the IPC were compared for all samples across the three kits.

MATERIALS AND METHODS

Degraded samples (N=15) were chosen based on varying DNA concentrations and degree of degradation. These samples included DNA from bones, decomposed tissue, and formalin-damaged tissues. Inhibited samples (N=100) were generated using male standard DNA (0.5ng/uL) spiked with aliquots of various concentrations of hematin, humic acid, calcium, melanin, and salt (NaCl).

All samples were quantified in duplicate using Quantifiler® Trio, PowerQuant, and InnoQuant using manufacturer's protocols. DNA quantification was performed on a 7500 real-time quantitative PCR (Life Technologies). Data were analyzed using the HID Real-Time PCR Analysis Software v1.2 and each kit's specific template. Additional analysis using the PowerQuant™ Analysis Tool v1 was required for the PowerQuant kit.

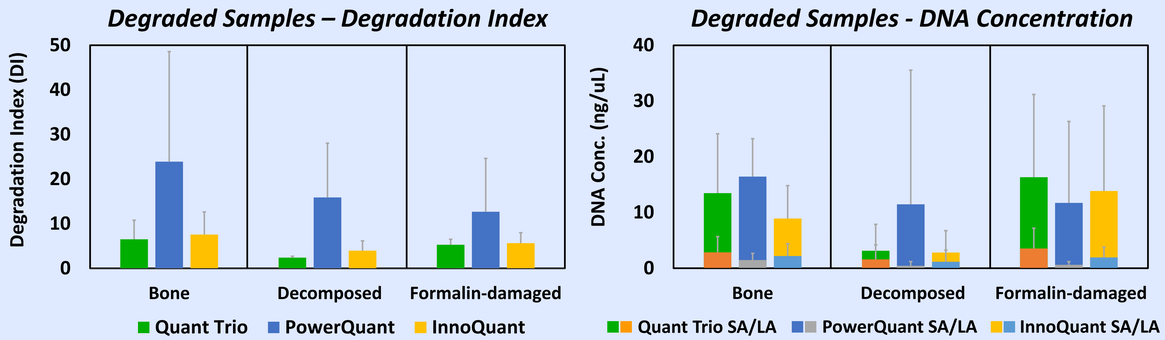

Figure 1. Average degradation indices (left) and average DNA concentrations of the small amplicons (SA) and large amplicons (LA; pictured right) for the degraded samples, including bone, decomposed tissue, and formalin-damaged tissues. Data presented as average ± SD. N = 5

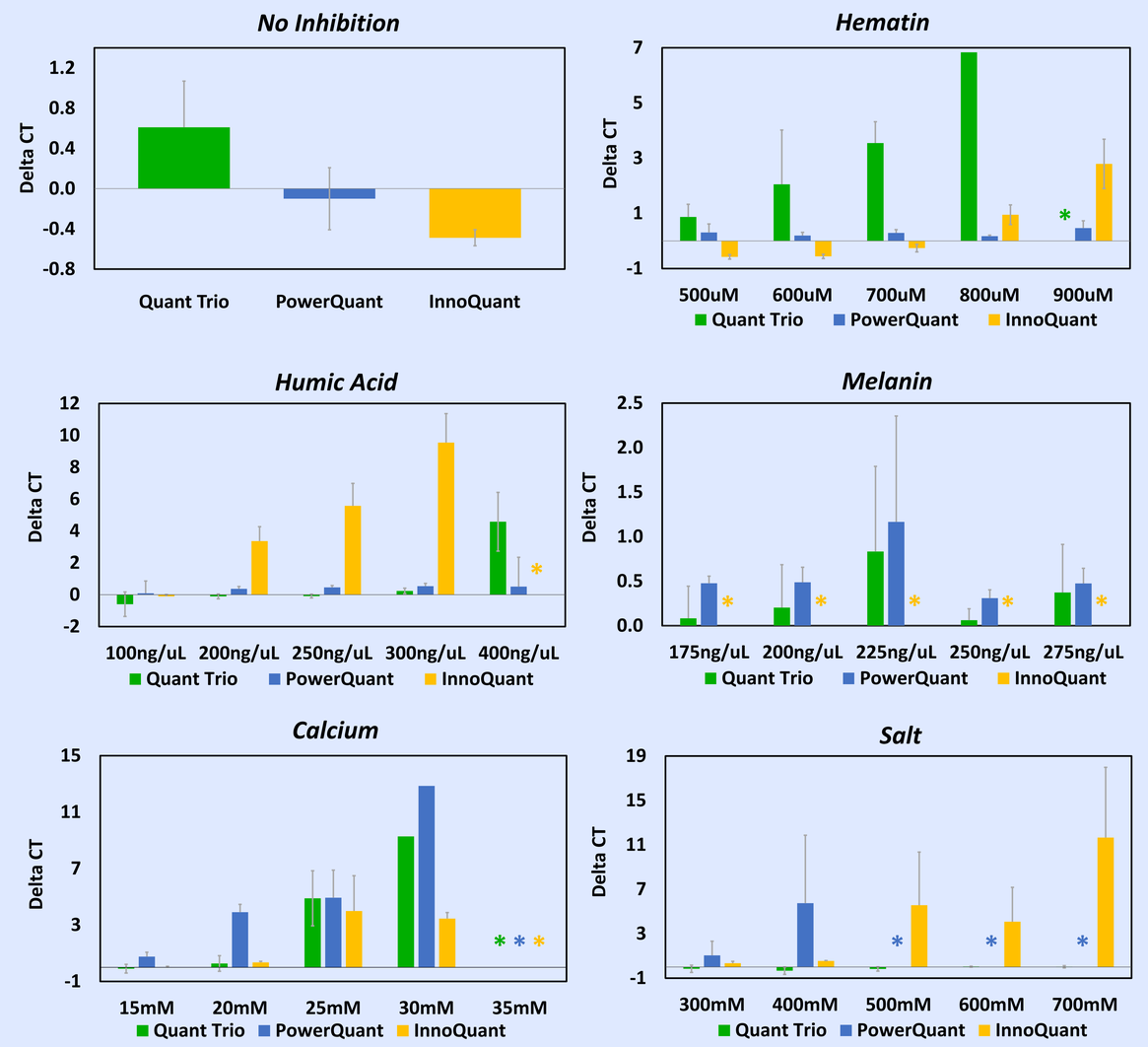

Figure 2. Average IPC values (delta CT) of the various inhibitors and concentrations generated for each commercial qPCR kit. An "" denotes an undetermined CT for that corresponding kit. Data presented as average ± SD. N = 4*

RESULTS AND DISCUSSION

- Quantitation values and degradation indices were generally comparable between the kits with the bone, decomposed and formalin-damaged samples; although the PowerQuant® kit generated notably higher DI values than the other two kits in all samples.
- Overall, Quantifiler® Trio was the most tolerant system to all inhibitors and their concentrations tested in this study.
- PowerQuant® was tolerant to all inhibitors except for calcium and salt.
- InnoQuant™ HY was the most susceptible to the very high concentrations of inhibitors tested in this study, melanin in particular.
- InnoQuant™ HY generally produced higher ΔC_T values and lower DNA concentrations than the other two kits, suggesting that this system may be less tolerant to extremely inhibited samples.

It should be noted that the performance of these kits with DNA samples that contain much lower concentrations of the inhibitors tested in this study may differ.

The use of a commercial qPCR kit that provides predictive information (DNA concentration, level of degradation and inhibition) about a sample is beneficial to any human identification application, such as forensic casework, missing persons or mass disaster situations. Knowledge about current commercial quantitative PCR kits' tolerances to sample degradation and extreme inhibition could aid in the success of downstream STR typing.

ACKNOWLEDGEMENTS

This study was partially supported by a Graduate Research Fellowship Award #2015-R2-CX-0029 (National Institute of Justice, Office of Justice Programs, U.S. Department of Justice). The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the author(s) and do not necessarily reflect those of the National Institute of Justice. The authors would also like to thank Life Technologies, Promega, and InnoGenomics Technologies for supplying their commercial qPCR kits that were used for this study.

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