

## Introduction

Quantifiler® Trio is commonly used in forensic DNA casework to quantify human DNA and target appropriately in short tandem repeat (STR) amplification. This kit analyzes one target on the Y chromosome, as well as large and small autosomal targets, thus giving useful information regarding male-female mixtures and degradation. It also contains an internal PCR control (IPC), which provides information on inhibition. Quantifiler® Trio evaluates sample quality using degradation and IPC information, in a metric called Quality Index (QI).

Reducing reaction volumes without reducing sample volume creates conditions more sensitive to inhibitors in the sample. This work evaluates the severity of the concept, and its correlation to inhibition during STR amplification with the Globalfiler® amplification kit.

## Objectives

- Compare IPC  $C_T$  using varying levels of inhibitors in full and reduced reaction volumes in Quantifiler® Trio to evaluate the magnitude of inhibition response
- Evaluate if inhibition (elevated IPC  $C_T$ ) in reduced volume of Quantifiler® Trio is reflective of inhibition (reduced peak heights) in Globalfiler®

## Materials and Methods

- Inhibited samples containing a constant concentration of DNA and 0 - 500 ng/ $\mu$ L humid acid were created and analyzed in Quantifiler Trio. Purchased DNA from an immortal cell line was used (2800M).
- Manufacturer's instructions and protocol were used and run simultaneously with our reduced volume method\* (see Table 1)
- Approximately 1.0 ng of selected samples was amplified in triplicate using the Globalfiler® amplification kit, according to manufacturer's recommendations

Quantifiler® Trio Components	Standard Protocol	Reduced Volume Protocol
Reaction Mix	10	2.5
Primer Mix	8	2
Sample	2	2
Total Volume	20	6.5

**Table 1. Reaction Volumes**

Volumes (in  $\mu$ L) used in manufacturer's protocol and our modified reduced volume protocol. Sample volume remained constant (2.0 $\mu$ L).

\*Applied Biosystems 7500 Real-Time PCR System and HID Real-Time PCR Analysis Software v 1.2 were used

## Results

- As expected, lower concentrations of inhibitor elevate IPC  $C_T$  in reduced volume compared to standard protocol (see Table 2)

## Results

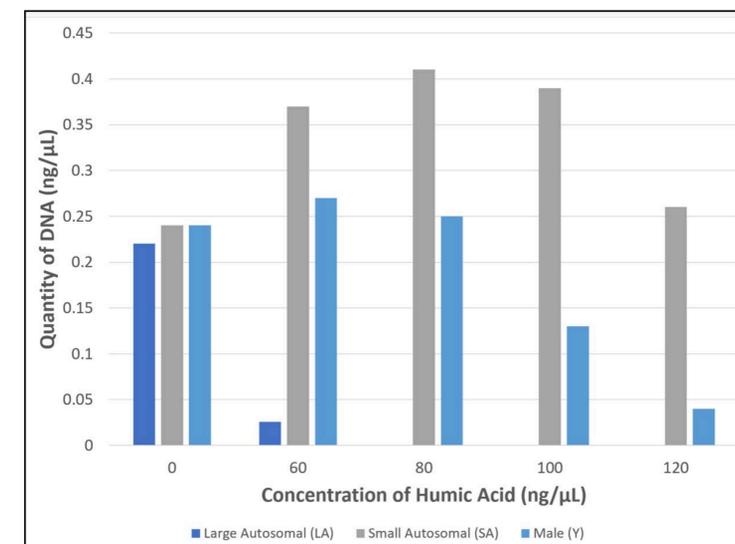
- Our reduced volume method detected mild and complete inhibition by elevated and undetected IPC  $C_T$ , respectively (see Table 2)
- Humic acid concentrations that produced elevated IPC  $C_T$  in our method produced no reduction in peak heights when amplified in Globalfiler®
- IPC and LA targets are more sensitive to humic acid inhibition than SA and Y targets (see Graph 1)
- At low inhibitor concentrations, small autosomal (SA) detection increases and reflects erroneously high quantities (see Graph 1)

Humic Acid Concentration (ng/ $\mu$ L)	Full		Reduced	
	500	120	100	80
500	ND	ND	ND	ND
120	27.46	ND	ND	ND
100	27.3	ND	ND	ND
80	27.46	ND	ND	ND
60	27.44	32.78	29.5	28.08
40	27.06	28.08	28.27	
20	27.3			
0	27.46			

**Table 2. Average IPC  $C_T$  values**

Columns 3 and 4 show the IPC  $C_T$  values in the full and reduced reactions, respectively.

Green represents samples with no inhibition (IPC  $C_T$  similar to the baseline of ~28 cycles). Yellow represents mild to moderate inhibition (elevated IPC  $C_T$ ), and red represents complete inhibition (IPC  $C_T$  not detected, ND)



**Graph 1. Calculated DNA quantities for varying levels of humic acid in reduced reaction volumes**

All samples contain the same amount of DNA. LA target shows severe response to inhibition at 60 ng/ $\mu$ L and complete inhibition at and above 80 ng/ $\mu$ L humic acid (HA). Male target shows little to no response to inhibition up to 100 ng/ $\mu$ L and severe response at 120 ng/ $\mu$ L HA. SA target demonstrates no inhibition; instead, similar or increased detection at all tested levels of HA. Inhibition of LA target (214 bases) occurs at lower inhibitor levels than smaller targets (Y, 75 bases and SA, 80 bases).

## Discussion and Future Work

- Our modified Quantifiler® Trio method can detect moderate and complete inhibition via elevated IPC  $C_T$  and undetected IPC  $C_T$ , respectively. These markers did not correlate to inhibition in Globalfiler® amplification.
- Reduction in reaction volume produces proportional increase of sensitivity to inhibition
- We plan to investigate the possibility that humic acid mechanism of inhibition causes preferential amplification of small autosomal and Y targets

## References

- Cho, Yoonjung, et al. "Validation of Reduced Reagent Volumes in the Implementation of the Quantifiler® Trio Quantification Kit." *Journal of Forensic Sciences*, vol. 63, no. 2, Wiley Subscription Services, Inc, 2018, pp. 517–25, <https://doi.org/10.1111/1556-4029.13578>.
- Westring, Christian G., et al. "Validation of Reduced-Scale Reactions for the Quantifiler™ Human DNA Kit." *Journal of Forensic Sciences*, vol. 52, no. 5, Blackwell Publishing Ltd, 2007, pp. 1035–43, <https://doi.org/10.1111/j.1556-4029.2007.00525.x>.
- ThermoFisher Scientific. Quantifiler HP and Trio DNA Quantification Kits User Guide. Revision H. 09 Oct 2018.