A Comparative Analysis of Quantifiler® Duo and Plexor®HY DNA Quantification Systems.

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Abstract

A comparative analysis of Quantifiler® Duo and Plexor®HY DNA quantification systems was conducted to assist forensic laboratories in choosing the combined human-male DNA quantification method that best meets their needs while identifying unique characteristics of each system. The systems evaluated were internally validated at Marshall University Forensic Science Center prior to the initiation of this study. This study consisted of an identical sample comparison of each system's standard curve quality metrics, sensitivity, precision, reproducibility, contamination, and mixture evaluation.

Introduction

The following validation studies were performed with each system:

Standard Curve Quality Metrics: Purpose was to establish quality metrics to be used in determination of pass/failure of a standard curve. Metrics analyzed were slope and R^2. Up to two poor standards could be removed.

Precision Study: Purpose was to assess how closely the C_T (The cycle number at which detected fluorescence passes a preset threshold) of a standard sample that was quantified multiple times matched each other.

Reproducibility Study: Purpose was to determine how closely the concentration values of mock casework samples were to each other when they were quantified multiple times.

Sensitivity Study: Purpose was to determine the lowest limits of DNA detection for each quantification system.

Mixture Analysis Study: Purpose was to determine how each quantification system performed with mixed samples of known mixture ratios and concentrations.

Contamination Study: Purpose was to determine if contaminating DNA would be detected in any Quantification Negative (QN) control samples included in the study.

Materials and Methods

Standard Curve Quality Metrics: A standard dilution series was created for each quantification system and analyzed multiple times by Real-Time PCR. The quality metrics from each standard curve combination was then compared to the recommended quality metrics published by the manufacturer.

Precision Study: Samples from the standard dilution series were quantified multiple times The reported C_T's for each dilution were then analyzed to calculate the average and Coefficient of Variance of each kit.

Reproducibility Study: Standard dilutions and "unknown" samples were quantified multiple times. The reported concentrations for each sample were then analyzed to calculate the average, standard deviation, and 95% confidence interval of each system.

Contamination Study: Quantification negative control samples (QN) included on each quantified plate as well as several additional QN samples were analyzed to determine if contaminating DNA was present.

Mixture Study: A male and female dilution series were mixed in inverse proportions. 1:1 mixtures of high, medium, and low concentration were also prepared. These samples were quantified in duplicate. The difference between the prepared concentration and the reported concentration of both the total human and human male component of each mixture was computed.

Sensitivity Study: An extended series of standard dilutions was made with each kit by continuing the dilution series out to three more dilutions beyond what was recommended by each system's protocol. After quantification, each of these dilutions was analyzed to determine if the quantification system could detect DNA and what concentration it determined for each dilution.

Results

Standard Curve Quality Metrics - Autosomal Detector				
	Quantifiler® DUO		Plexor®HY	
	Slope	R^2	Slope	R^2
Requirements	-3.0 to -3.6	≥0.99	-3.3 to -4.0	≥0.99
AVG	-3.260	0.994	-3.769	0.995
MIN	-3.418	0.990	-4.170	0.990
MAX	-3.077	0.998	-3.590	0.998

Table 1: Autosomal Standard Curve Quality Metrics Data.

Standard Curve Quality Metrics - Male Detector				
	Quantifiler® DUO		Plexor®HY	
	Slope	R^2	Slope	R^2
Requirements	-3.0 to -3.6	≥0.99	-3.0 to -3.6	≥0.99
AVG	-3.316	0.992	-3.454	0.998
MIN	-3.479	0.989	-3.530	0.996
MAX	-3.096	0.996	-3.290	0.999

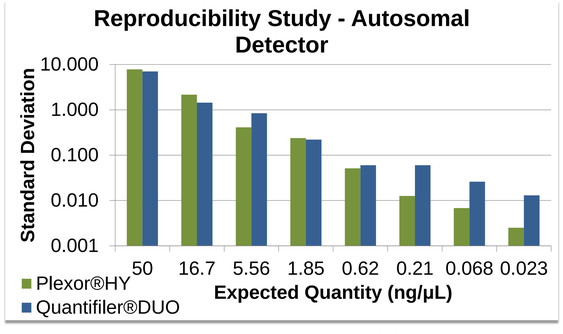
Table 2: Male Standard Curve Quality Metrics Data.

Precision Comparison Study – Autosomal Detector					
Plexor®HY			Quantifiler® DUO		
Standard (ng/µL)	AVG CT	CV	Standard (ng/µL)	AVG CT	CV
50	18.55	0.52%	50	23.58	0.72%
10	21.39	0.75%	16.7	25.23	0.48%
2	24.14	0.49%	5.56	26.88	0.71%
0.4	26.76	0.92%	1.85	28.63	0.66%
0.08	29.28	0.45%	0.62	29.94	0.37%
0.016	31.88	1.50%	0.21	31.33	0.96%
0.0032	34.85	3.43%	0.068	32.99	1.21%
			0.023	34.61	1.73%

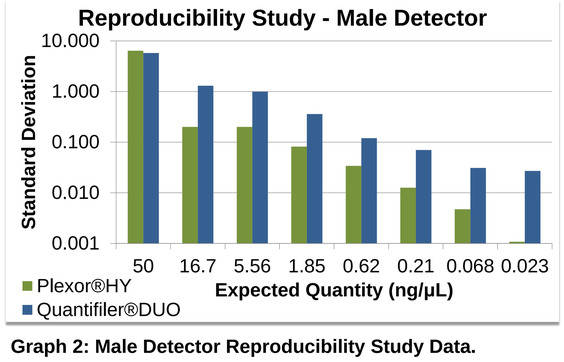
Table 3: Autosomal Detector Precision Study Data.

Precision Comparison Study – Male Detector					
Plexor®HY			Quantifiler® DUO		
Standard (ng/µL)	AVG CT	CV	Standard (ng/µL)	AVG CT	CV
50	19.38	1.66%	50	23.36	1.11%
10	21.51	1.48%	16.7	24.98	0.92%
2	23.94	0.97%	5.56	26.62	1.05%
0.4	26.38	1.07%	1.85	28.26	0.81%
0.08	28.74	1.62%	0.62	29.79	0.97%
0.016	31.31	0.71%	0.21	31.32	1.09%
0.0032	33.85	2.63%	0.068	32.83	0.97%
			0.023	34.48	1.68%

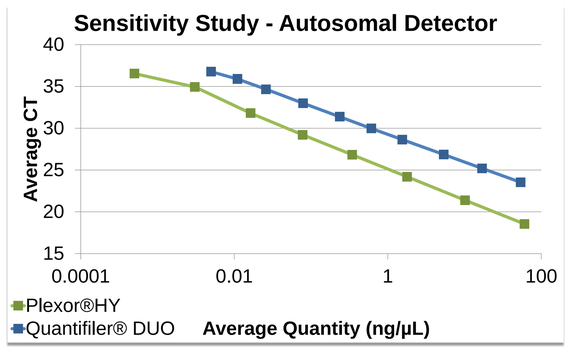
Table 4: Male Detector Precision Study Data.



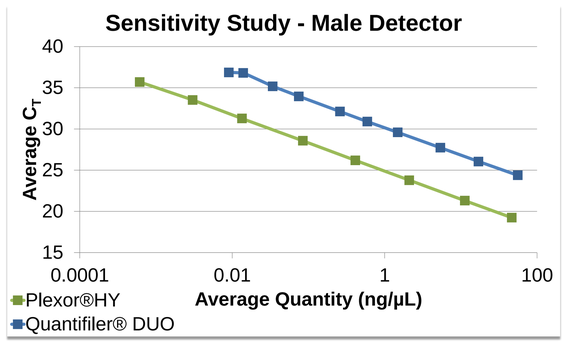
Graph 1: Autosomal Detector Reproducibility Study Data.



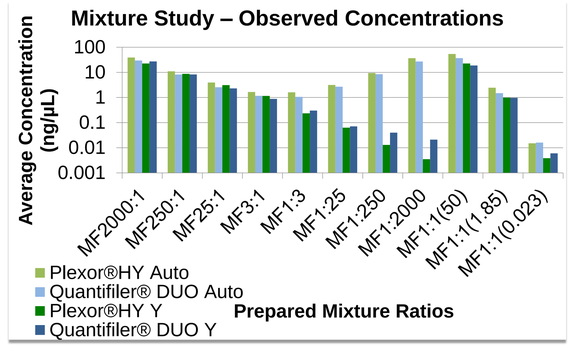
Graph 2: Male Detector Reproducibility Study Data.



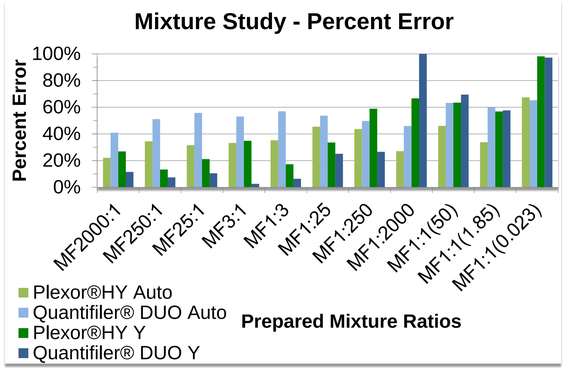
Graph 3: Autosomal Detector Sensitivity Study Data. Higher C_T correlates with a lower concentration.



Graph 4: Male Detector Sensitivity Study Data. Higher C_T correlates with a lower concentration.



Graph 5: Mixture Study Data for both detectors



Graph 6: Percent Error of observed sample concentrations from expected sample concentrations

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Discussion

Standard Curve Quality Metrics Study: All Quantifiler® DUO Standard Curves passed. All Plexor®HY Standard Curves passed except one, which exhibited a slope less than -4.0. Sample concentrations calculated with this standard curve were found to have no significant impact on concentration calculations.

Precision Study: Both quantification systems displayed greater C_T precision in the male detector over the autosomal detector. Precision with both quantification systems decreased with lower concentration samples. Quantifiler® DUO displayed greater precision than Plexor®HY.

Reproducibility Study: Both quantification systems displayed lower reproducibility with higher concentration samples and greater reproducibility with lower concentration samples. Plexor®HY displayed more reproducibility than Quantifiler® DUO.

Sensitivity Study: Plexor®HY displayed greater sensitivity than Quantifiler® DUO. Concentrations reported for these low level samples tended to vary greatly from the expected concentration.

Mixture Study: Plexor®HY displayed less deviation from the expected concentration in the autosomal channel. Quantifiler® DUO displayed less deviation from the expected concentration in the male channel.

Contamination Study: Quantifiler® DUO showed no contamination in any QN sample. Plexor®HY showed contamination in 1 QN sample (0.00022 ng/µL in the Male Channel only). The other 5 QN samples on the same 96-well plate were clean. It was concluded that the

Conclusions

Both kits displayed unique benefits and strengths.

Two-source Male:Female mixtures quantified with Quantifiler® DUO can be resolved to estimate how much DNA came from each contributor. Plexor®HY primers have multiple binding sites on each target loci, so the amount of DNA coming from the female contributor cannot be estimated.

Plexor®HY was able to detect samples of extremely low concentrations. The lowest concentration detected by Plexor®HY was a 0.00064 ng/µL standard dilution and the lowest concentration detected by Quantifiler® DUO was a 0.0025 ng/µL. Future studies include a study to determine the lowest concentration that a full or partial DNA profile can be reasonably expected to be developed with each kit. The addition of the MavenQST™ quantification system to the comparison study is also planned after internal validation is completed.

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