Comparative Analysis of the Quantifiler® Duo, Quantifiler® Trio, and Investigator® Quantiplex HYres DNA Quantification Kits

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Abstract

The ultimate goal was to identify which of the three kits would best suit the needs of the laboratory by looking at the statistical results and overall outcomes of the previously studies performed. In order to determine this, it was imperative to show that each of the kits were robust and reliable, and would therefore have the potential to be implemented for casework. After this was determined, the kits could then be compared in the areas thought to be of high importance to the West Virginia State Police Forensic Laboratory's (WVSPFL) Biochemistry Section.

Introduction

This project focused on comparing the Life Technologies® Quantifiler® Trio DNA Quantification kit and the Qiagen® Investigator® Quantiplex HYres kit, with the Life Technologies® Quantifiler® Duo DNA Quantification kit, which is the kit currently validated by the WVSPFL Biochemistry Section. The following studies were performed with all three kits in order to directly evaluate their capabilities in a forensic laboratory setting: standard curve quality metrics, accuracy, precision, sensitivity, repeatability, stochastic, known/non-probative, mixture, and contamination. Statistical calculations were performed on the concentration and threshold cycle (C_T) results for the samples in each study in order to evaluate the kits' performances and allow for comparisons between them. The ultimate goal was to identify which of these three kits would best serve the needs of the WVSPFL based on statistical results and overall outcomes of the previously mentioned studies.

Methods

Standard Curve Quality Metrics: Twelve Quantiplex HYres, twelve Quantifiler® Trio, and ten Quantifiler® Duo sets of standards were made on three respective 96-well plates. The curves were generated by analyzing two columns of adjacent standards each. Either of the two data points used to generate the curve at any one concentration may be omitted to meet the required values.

Accuracy and Precision: An extract of a buccal swab was selected, serial dilutions were made, and each of these dilutions were quantified using the three kits. Since Quantifiler® Duo is the validated kit currently in use by the WVSPFL Biochemistry Section, its values were used as the standard (expected value) with which to compare the other two kits (unknown value).

Sensitivity and Stochastic: The sensitivity and stochastic studies were conducted by taking a serial dilution of the 2800M amplification control beyond the concentration ranges of the kits to determine if they were able to detect DNA at extremely low quantities. The values chosen were 0.005, 0.0025, 0.0005 and 0.00025 ng/µL.

Known and Non-Probative: Known and non-probative samples were created to simulate casework samples, as well as inhibited and degraded samples.

Mixture: Mixtures of set ratios were created using a male and a female contributor to determine if the quantification kits could detect male DNA at varying concentrations, specifically in the presence of a high amount of female DNA.

Repeatability: This was tested by running each study, when adequate sample was available, in triplicate on each quantification plate.

Contamination: Contamination was evaluated with the use of Known Reagent Controls (KRC's), consisting of all reagents included in a reaction; no sample or DNA was added.

Results

Standard Curve Quality Metrics

Table 1: Results of the Autosomal Target

Standard Curve Quality Metrics- Autosomal Target						
	Quantifiler® Duo		Quantifiler® Trio		Investigator® Quantiplex Hyres	
	Slope	R^2	Slope	R^2	Slope	R^2
Requirements	-3.0 to -3.6	≥0.98	-3.0 to -3.6	≥0.99	-3.0 to -3.6	≥0.99
Max.	-3.13276	0.996829	-3.176	1	-3.291	0.999
Min.	-3.35792	0.991192	-3.521	0.999	-3.48	0.998
Average	-3.2249842	0.995	-3.25767	0.999	-3.388	0.999

Table 2: Results of the Male Target

Standard Curve Quality Metrics- Male Target						
	Quantifiler® Duo		Quantifiler® Trio		Investigator® Quantiplex Hyres	
	Slope	R^2	Slope	R^2	Slope	R^2
Requirements	-3.0 to -3.6	≥0.98	-3.0 to -3.6	≥0.99	-3.0 to -3.6	≥0.99
Max.	-3.151307	0.997825	-3.2	0.999	-3.229	0.999
Min.	-3.35792	0.987206	-3.358	0.996	-3.362	0.998
Average	-3.2368554	0.994	-3.28333	0.999	-3.284	0.999

While all three kits met the manufacturer's requirements with all of the standard curves generated, Quantifiler® Duo had a lower average R^2 value when compared to the Quantifiler® Trio and Quantiplex Hyres. All three kits had an average slope near -3.3 and there did not appear to be any significant differences between the kits, in regards to slope.

Accuracy and Precision

Table 3: Human Target Accuracy Statistics

Accuracy-Human Target Average Concentration and Difference between Desired and Actual Concentration						
Desired Concentration (ng/µL)	Quantifiler® Duo		Quantifiler® Trio		Investigator® Quantiplex Hyres	
	Average (ng/µL)	Difference (ng/µL)	Average (ng/µL)	Difference (ng/µL)	Average (ng/µL)	Difference (ng/µL)
14.643	14.323	0.320	11.053	3.590	12.679	1.964
9.000	8.790	0.210	6.970	2.030	8.230	0.770
3.000	2.633	0.367	2.545	0.455	2.559	0.441
1.000	0.722	0.278	0.837	0.163	0.690	0.310
0.050	0.0390	0.011	0.0434	0.00660	0.030	0.020

Table 4: Human Target Precision Statistics

Precision- Human Target Average, Standard Deviation, and Relative Standard Deviation of Concetrations									
Desired Concentration (ng/µL)	Quantifiler® Duo			Quantifiler® Trio			Investigator® Quantiplex Hyres		
	Average (ng/µL)	Std. Dev.	RSD (%)	Average (ng/µL)	Std. Dev.	RSD (%)	Average (ng/µL)	Std. Dev.	RSD (%)
14.643	14.323	1.662	11.604	11.053	0.981	8.878	12.679	1.973	15.559
9.000	8.790	0.503	5.725	6.970	0.233	3.346	8.230	0.331	4.027
3.000	2.633	0.400	15.191	2.545	0.166	6.534	2.559	0.131	5.112
1.000	0.7223	0.0449	6.210	0.8367	0.0356	4.260	0.6897	0.0619	8.976
0.050	0.03900	0.00964	24.727	0.04340	0.00282	6.489	0.03000	0.00265	8.819

The quantification values produced by Quantifiler® Trio and Quantiplex HYres are not statistically significantly different from those produced by Quantifiler® Duo for the same sample. Quantifiler® Trio resulted in the lowest standard deviation and RSD percentage with the majority of the concentrations. These results are also reflected in the C_T values' calculations.

Sensitivity and Stochastic

Table 5: Human Target Concentration (ng/µL) Results

Sensitivity- Human Target Average Concentration and Standard Deviation						
Targeted Concentration	Quantifiler® Duo		Quantifiler® Trio		Investigator® Quantiplex Hyres	
	Average (ng/µL)	Standard Deviation	Average (ng/µL)	Standard Deviation	Average (ng/µL)	Standard Deviation
0.005	0.00209	N/A	0.00113	0.0000535	---	N/A
0.0025	0.00326	N/A	0.000946	0.000321	0.000243	0.0000216
0.0005	---	N/A	---	N/A	---	N/A
0.00025	---	N/A	---	N/A	---	N/A

Table 6: IPC Target CT Results

Sensitivity- IPC Target Average and Standard Deviation of Ct Values						
Targeted Concentration	Quantifiler® Duo		Quantifiler® Trio		Investigator® Quantiplex Hyres	
	Average	Standard Deviation	Average	Standard Deviation	Average	Standard Deviation
0.005	28.300	0.0656	27.366	0.1647	29.843	0.1604
0.0025	27.920	0.4915	27.209	0.1205	30.229	0.1848
0.0005	28.233	0.2495	27.109	0.1292	30.174	0.1602
0.00025	28.367	0.1563	27.184	0.2165	29.726	0.6582

Quantifiler® Trio was the only kit to produce quantities for all three repeats of the first two dilutions in the series. While it was not able to detect DNA in the last two dilutions, these samples did not result in any usable information from amplification and CE.

Known and Non-Probative

- Quantification results of known and non-probative samples were as expected for their respective sample types
- All kits quantified samples similarly
- Electropherograms for known samples contained all expected alleles.
- Electropherograms for questioned samples resulted in full profiles with no indication of dropout.

Table 7: Human Target Accuracy Statistics

Inhibition/Degradation- Human Target Average Concentrations (ng/µL) and Standard Deviation of Concentration						
Sample	Quantifiler® Duo		Quantifiler® Trio		Investigator® Quantiplex Hyres	
	Average	Standard Deviation	Average	Standard Deviation	Average	Standard Deviation
ID_Awcontrol	6.160	0.473	8.269	0.714	10.846	1.053
Inhib_BeforeDyeAW	3.963	0.629	5.515	0.300	8.725	0.478
Inhib_AfterDyeAW	Blank	N/A	Blank	N/A	Blank	N/A
Deg_NeatBleachAW	2.577	0.192	3.690	0.067	5.129	0.439
Deg_DilBleachAW	2.873	0.335	4.115	0.125	5.714	0.164
ID_HBMcontrol	11.177	0.931	10.527	0.976	10.473	0.212
Inhib_BeforeSoilHBM	9.520	0.871	13.293	0.387	14.197	0.243
Inhib_AfterSoilHBM	1.737	0.195	7.627	0.440	5.351	0.056
Deg_NeatElimHBM	13.870	0.632	12.864	0.324	14.742	1.237
Deg_DilElimHBM	11.957	0.928	13.119	0.653	13.890	0.609
ID_JTHcontrol	8.573	1.204	17.907	1.597	16.500	0.207
Inhib_BeforeRiverJTH	7.527	0.263	15.969	0.908	16.798	0.477
Inhib_AfterRiverJTH	5.243	0.305	10.467	0.121	10.983	0.431
Deg_15UVJTH	4.250	0.624	8.724	0.441	10.239	0.378
Deg_5UVJTH	5.377	0.439	11.034	0.319	12.261	0.152
Deg_KRC	Blank	N/A	0.000	0.000	Blank	N/A
Inhib_KRC	Blank	N/A	0.000	N/A	Blank	N/A

Table 8: Human Target Accuracy Statistics

Sample	CE Results
ID_Awcontrol	All peaks present.
Inhib_BeforeDyeAW	All peaks present, no indication of inhibition for any BeforeDye sample.
Inhib_AfterDyeAW	No peaks called.
Deg_NeatBleachAW	All peaks present, no indication of degradation for and NeatBleach sample.
Deg_DilBleachAW	All peaks present, no indication of degradation for any of the DilBleach samples.
ID_HBMcontrol	All peaks present.
Inhib_BeforeSoilHBM	All peaks present, no indication of inhibition.
Inhib_AfterSoilHBM	Partial profile-5 loci with peaks called.
Deg_NeatElimHBM	All peaks present, no indication of degradation for any NeatElim sample.
Deg_DilElimHBM	All peaks present, no indication of degradation for any of the DilElim samples.
ID_JTHcontrol	All peaks present.
Inhib_BeforeRiverJTH	All peaks present, no indication of inhibition for any BeforeRiver sample.
Inhib_AfterRiverJTH	All peaks present, no indication of inhibition.
Deg_15UVJTH	All peaks present. Blue and Yellow dye channels indicate potential degradation.
Deg_5UVJTH	All peaks present. Blue dye channel indicates potential degradation.
Inhib_KRC	No peaks called.
Deg_KRC	No peaks called.
Inhib_1:10	No peaks called.
Inhib_1:50	All peaks present.
Inhib_1:100	All peaks present.

The inhibited samples' IPC results did not indicate inhibition occurred, with the exception of the sample with indigo dye added after extraction. The degradation indexes were not significantly higher than their controls, despite quantification values. The capillary electrophoresis results did indicate some potential degradation in certain dye channels; however it was not enough to significantly interfere with interpretation of the profiles.

Mixture

Table 9: Mixture Study Results

Mixture- Average Concentrations and Calculated M:F Ratio				
Targeted Ratio	Quantifiler® Duo			
	Human (ng/µL)	Male (ng/µL)	Calculated Female (ng/µL)	Calculated Ratio
8:1	0.103	0.082	0.0210	3.9:1
4:1	0.094	0.077	0.0170	4.5:1
2:1	0.074	0.041	0.0327	1.3:1
1:1	0.061	0.033	0.0280	1.2:1
1:2	0.070	0.032	0.0373	1:1.2
1:4	0.061	0.015	0.0460	1:3.1
1:8	0.057	0.016	0.0413	1:2.6
Targeted Ratio	Quantifiler® Trio			
	Human (ng/µL)	Male (ng/µL)	Calculated Female (ng/µL)	Calculated Ratio
8:1	0.068	0.085	-0.0169	-5.0:1
4:1	0.057	0.060	-0.0031	-19.3:1
2:1	0.054	0.050	0.0045	11.0:1
1:1	0.052	0.035	0.0176	2.0:1
1:2	0.066	0.037	0.0290	1:0.8
1:4	0.046	0.021	0.0250	1:1.2
1:8	0.059	0.017	0.0414	1:2.4
Targeted Ratio	Investigator® Quantiplex Hyres			
	Human (ng/µL)	Male (ng/µL)	Calculated Female (ng/µL)	Calculated Ratio
8:1	0.086	0.051	0.0353	1.4:1
4:1	0.079	0.041	0.0381	1.1:1
2:1	0.079	0.038	0.0409	0.9:1
1:1	0.082	0.030	0.0513	0.6:1
1:2	0.081	0.023	0.0578	1:2.5
1:4	0.080	0.017	0.0625	1:3.6
1:8	0.087	0.009	0.0781	1:9.1

The calculated mixtures did not reflect the expected results; however when the mixtures were calculated based on the RFU values, they were relatively close to the targeted values, indicating the ratios were made correctly.

Repeatability

Table 10: Known/Non-Probative Statistics Used for Repeatability

Known/Non Probative--Quantification Concentrations and Standard Deviations												
Sample	Quantifiler® Duo				Quantifiler® Trio				Investigator® Quantiplex Hyres			
	Set 1 (ng/µL)	Set 2 (ng/µL)	Set 3 (ng/µL)	Std. Dev.	Set 1 (ng/µL)	Set 2 (ng/µL)	Set 3 (ng/µL)	Std. Dev.	Set 1 (ng/µL)	Set 2 (ng/µL)	Set 3 (ng/µL)	Std. Dev.
K_JCH	1.040	0.968	1.210	0.124	0.677	0.772	0.784	0.059	1.256	0.896	0.893	0.209
K_SK	4.470	4.060	6.660	1.398	5.374	5.619	5.103	0.258	6.714	5.136	5.364	0.853
Q_CigFilter	0.151	0.166	0.179	0.014	0.093	0.108	0.119	0.013	0.148	0.167	0.165	0.010
Q_MAChair	1.110	1.380	1.470	0.187	0.757	0.762	0.676	0.048	0.811	0.626	0.855	0.122
Q_SM-blood	0.084	0.084	0.083	0.001	0.047	0.058	0.059	0.006	0.069	0.060	0.080	0.010
Q_SM-saliva	0.265	0.355	0.387	0.063	0.246	0.296	0.298	0.029	0.283	0.324	0.345	0.032

Contamination

- All KRC's quantified at 0.000 ng/µL
- Either "undefined" or rounded to 0.000 ng/µL

Conclusion

Regarding the internal validation for Quantiplex HYres and Quantifiler® Trio, it was able to be demonstrated that both quantification kits are robust and reliable and have the potential to be implemented by the WVSPFL Biochemistry Section for casework. It was demonstrated though the mixture study that all kits were able to detect a male component with a high concentration of female DNA present, which was of high importance to the WVSPFL. It can also be concluded that the results of all samples used during these studies can be considered free of contamination due to all KRC's demonstrating a lack of DNA present. The sensitivity and stochastic study highlighted Quantifiler® Trio's potential to detect extremely low concentrations of DNA that the other two quantification kits in this study could overlook. The precision and reproducibility studies also demonstrated an aspect of Quantifiler® Trio's abilities, showing how reliable and precise the kit is when tested numerous times, compared to the other quantification kits involved. While the results of these studies will play a large factor in helping the WVSPFL Biochemistry Section make a decision regarding quantification kit implementation in the future, many other aspects must also be considered. Future studies, including additional sensitivity and concordance studies, will be performed prior to the laboratory's final decision.

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