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## **VIRGINIA DEPARTMENT OF FORENSIC SCIENCE**

### **VALIDATION OF THE QUANT STUDIO 5 SYSTEM**

**Draft v.6**

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#### **PURPOSE**

This validation study assessed the performance of the PowerQuant® DNA Quantification System in conjunction with the QuantStudio™ 5 Real-Time PCR Instrument in quantitating single source, sensitivity series, male:female and male:male mixtures, degraded, mock casework and non-probative casework samples. These quantitation data sets were compared to the Plexor™ HY System used in conjunction with the Stratagene MX3005P real-time PCR instrument to determine if the expected quantitation results were achieved.

#### **MATERIALS AND METHODS**

##### ***Sample preparation***

Mixture and sensitivity samples were prepared using two different DNA extraction techniques. Tissue samples were extracted using phenol/chloroform followed by Microcon® purification, as described in the Virginia Department of Forensic Science (VDFS) procedures manual.<sup>1</sup> Buccal or blood samples were extracted using the DNA IQ™ System manually, also as described in the VDFS procedures manual.<sup>1</sup>

##### ***Sensitivity series***

In order to establish the sensitivity range needed for testing, a basic assessment of the system was done. All samples were originally quantitated in singlicate on the QuantStudio™ 5 instrument (QS5) using the PowerQuant® system (PQ). Based on the total autosomal results, two separate sensitivity experiments were conducted. In each experiment, all samples were prepared in triplicate and quantitated on two different days.

##### ***Mixture series***

The first experiment compared the QS5 System to the Applied Biosystems 7500 Real Time PCR instrument (7500). DNA samples were quantitated in triplicate. For all other experiments, DNA samples were initially quantitated as single source samples and then again after the mixtures were created. A dilute male DNA sample was held constant and mixed with an increasingly concentrated female sample. Mixture samples were quantitated on the QS5 with PQ on two separate days in singlicate.

##### ***Non-probative casework***

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<sup>1</sup> Virginia Department of Forensic Science Forensic Biology Procedures Manual, Extraction of DNA.

Seventeen casework samples that were potentially good candidates for Y-STR analysis were retrieved from the Normalization Wizard normalization plate. Candidacy was determined by the presence of sufficient DNA extract volume left in Normalization plate and Y-DNA quantitation data. If there was no Y-DNA quantitation data, the samples were not collected for this test. These samples were then manually setup for quantitation and analyzed on the QS5 platform. The samples had been normalized based upon the autosomal target of 0.1 ng/μL, therefore, the expected autosomal quantitation values for the PQ/QS5 analysis were the same. The concentration of expected Y-DNA was estimated for the PQ/QS5 system based upon the initial Y-DNA concentration measured using PlexorHY™ and the Stratagene MX3000P qPCR instrument (PLX) and the DNA dilutions performed by the Normalization wizard.

### ***DNA quantitation***

Reaction setup was performed using the PQ system following the manufacturer's recommendations.<sup>2</sup> Amplification used 2μL of DNA extract added to the 18μL amplification cocktail. Samples were then quantified using the QS5. The dye filters used on the QS5 were; FAM for autosomal target (84-base-pair amplicon), CAL Fluor Gold 540 (CFG540) for the male DNA targets (81bp and 136bp), TMR for the internal positive control (IPC) (435bp), Quasar 670 for degradation (294bp), and CXR for the passive reference dye. The raw data were collected with ABI QuantStudio Design & Analysis Software v 1.4.3 and analyzed using Promega's PowerQuant™ Analysis Tool software, version 1.0.0.0. Values are reported in ng/μL. Undetermined means the DNA value could not be detected.

### ***STR typing***

STR typing using PowerPlex® Fusion and Yfiler™ half-reaction volumes was performed following the Virginia Department of Forensic Science (VDFS) procedures manual. PowerPlex® Fusion and Yfiler™ half-reaction amplification reactions used 5 μL of DNA extract added to 7.5μL of amplification cocktail, unless otherwise indicated. Fragments were separated using a 3500xL Genetic Analyzer, (Applied Biosystems,) the raw data was collected with ABI Data Collection Software and analyzed using the GeneMapper®ID-X (GMIDX) software (Applied Biosystems) according to the VDFS procedures manuals.<sup>3,4</sup>

## **STATISTICAL ANALYSIS**

Statistical analyses were performed using Excel Microsoft Office Professional Plus, 2016.

## **RESULTS**

### ***Sensitivity studies***

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<sup>2</sup> Promega's Technical Manual for PowerQuant® System, 1/2020.

<sup>3</sup> Virginia Department of Forensic Science Forensic Biology Procedures Manual, POWERPLEX® FUSION Amplification and Long Term Storage.

<sup>4</sup> Virginia Department of Forensic Science Forensic Biology Procedures Manual, AMPFLSTR™ YFILER™ Amplification and Long Term Storage.

In order to establish the sensitivity range needed for testing, a basic assessment of the system was done. All samples were originally quantitated in singlicate on the QS5. Based on the total autosomal results, two separate sensitivity experiments were conducted. In each experiment, all samples were quantitated in triplicate.

The first experiment compared four single source DNA samples, two male (one buccal and one blood sample) and two female (both buccal samples), in serial dilutions. The serial dilution range started at 30ng/μL and ended at 0.00385 ng/μL. However, because the blood total autosomal quant was significantly lower than the buccal swabs, this sample's serial dilution range started at 3.75 ng/μL and ended at 0.00385 ng/μL. Table 1 displays the autosomal DNA quantitation results, while Table 2 displays the male [Y] DNA results. The estimates for both autosomal and Y-DNA targets deviated substantially from the expected values once the concentration target was below 0.118 ng/μL, so the experiment was repeated.

The second experiment consisted of four single source male DNA samples, all buccal samples, in serial dilution, quantitated in triplicate. The data in Table 3 shows the male autosomal DNA, while Table 4 shows the male [Y] DNA. The data demonstrated that both the Autosomal and Y-DNA concentrations were similar to expected values, although generally slightly lower, including those concentration below 0.118 ng/μL, suggested that an error was made in creating the less concentrated samples from the first sensitivity experiment. The percent CV was below 10% for autosomal DNA concentrations above 0.0295 ng/μL, except for the 0.475 ng/μL sample, M1. One of the replicates for M1 was much higher than the other two values, which were close to each other (0.8654 versus 0.6016 and 0.6229). This had the effect of raising the percent CV to 21.0357%.

The results for the Y-DNA target were similar. However, two different concentrations of the M1 sample showed %CV values above 10% at DNA concentrations greater than 0.0295 ng/μl; the 0.475 ng/μl sample and the 0.059 ng/μl sample produced %CV values of 24.5872% and 12.4243% respectively. The same issue as described above produced the larger %CV with one of the three replicates deviating from the other two.

**Table 1. Autosomal DNA Sensitivity Series Quantitation Data performed using QuantStudio 5.**

Sample name	Dilution	[AUTO]	Sample name	Dilution	[AUTO]	Sample name	Dilution	[AUTO]	Sample name	Dilution	[AUTO]
F2_30ng/μl		36.295532	F3_30ng/μl		33.7145538	M2_30ng/μl		38.7109489			
F2_30ng/μl		32.738174	F3_30ng/μl		33.9083862	M2_30ng/μl		33.3844528			
F2_30ng/μl		31.899632	F3_30ng/μl		35.9464111	M2_30ng/μl		35.4440804			
<b>AVG</b>		<b>33.644446</b>			<b>34.5231171</b>			<b>35.846494</b>			
<i>STDV</i>		2.333877			1.2364131			2.6859529			
%CV		6.9368865			3.5814062			7.4929305			
F2_15ng/μl		17.710918	F3_15ng/μl		19.3048267	M2_15ng/μl		15.4257565			
F2_15ng/μl		16.485605	F3_15ng/μl		20.9263535	M2_15ng/μl		16.6620121			
F2_15ng/μl		17.793644	F3_15ng/μl		22.1849861	M2_15ng/μl		17.2449512			
<b>AVG</b>		<b>17.330056</b>			<b>20.8053888</b>			<b>16.4442399</b>			
<i>STDV</i>		0.732484			1.4438850			0.9289435			
%CV		4.2266713			6.9399568			5.6490508			
F2_7.5ng/μl		8.6844864	F3_7.5ng/μl		10.1321011	M2_7.5ng/μl		7.30009985			
F2_7.5ng/μl		9.0227118	F3_7.5ng/μl		8.5776033	M2_7.5ng/μl		7.34726477			
F2_7.5ng/μl		9.7532825	F3_7.5ng/μl		11.1960382	M2_7.5ng/μl		8.64347839			
<b>AVG</b>		<b>9.1534936</b>			<b>9.9685809</b>			<b>7.76361434</b>			
<i>STDV</i>		0.5462684			1.3168540			0.7623495			
%CV		5.9678684			13.2100449			9.8195174			
F2_3.75ng/μl		4.3175197	F3_3.75ng/μl		4.1175733	M2_3.75ng/μl		4.1983633	M4_3.75ng/μl		3.873716354
F2_3.75ng/μl		4.0957742	F3_3.75ng/μl		4.6015496	M2_3.75ng/μl		4.32150841	M4_3.75ng/μl		4.577355862
F2_3.75ng/μl		4.1151395	F3_3.75ng/μl		5.5448203	M2_3.75ng/μl		4.09215403	M4_3.75ng/μl		4.242337227
<b>AVG</b>		<b>4.1761444</b>			<b>4.7546477</b>			<b>4.20400858</b>			<b>4.231136481</b>
<i>STDV</i>		0.1228168			0.7258359			0.1147814			0.35195345
%CV		2.9409139			15.2658194			2.7302836			8.31817769
F2_1.9ng/μl		2.2628937	F3_1.9ng/μl		2.5383904	M2_1.9ng/μl		1.91038513	M4_1.9ng/μl		1.763925195
F2_1.9ng/μl		2.2775962	F3_1.9ng/μl		2.5988045	M2_1.9ng/μl		2.1236279	M4_1.9ng/μl		2.309500933
F2_1.9ng/μl		2.2564702	F3_1.9ng/μl		2.7829745	M2_1.9ng/μl		2.08203173	M4_1.9ng/μl		1.773419142
<b>AVG</b>		<b>2.2656534</b>			<b>2.6400565</b>			<b>2.03868159</b>			<b>1.948948423</b>
<i>STDV</i>		0.01083			0.1274034			0.1130378			0.31228371
%CV		0.4780085			4.8257849			5.5446523			16.0231903
F2_0.95ng/μl		1.089358	F3_0.95ng/μl		1.3107235	M2_0.95ng/μl		1.02165723	M4_0.95ng/μl		0.933155537
F2_0.95ng/μl		1.2069747	F3_0.95ng/μl		1.3063911	M2_0.95ng/μl		1.04447711	M4_0.95ng/μl		1.145100713
F2_0.95ng/μl		1.1520542	F3_0.95ng/μl		1.1535821	M2_0.95ng/μl		1.2157892	M4_0.95ng/μl		1.058004379
<b>AVG</b>		<b>1.1494623</b>			<b>1.2568989</b>			<b>1.09397451</b>			<b>1.04542021</b>
<i>STDV</i>		0.0588512			0.0895012			0.1061099			0.1065315
%CV		5.1198902			7.1207963			9.6994811			10.1903042

F2_0.475ng/μl	0.5312386	F3_0.475ng/μl	0.5527787	M2_0.475ng/μl	0.47674805	M4_0.475ng/μl	0.459742665
F2_0.475ng/μl	0.4919352	F3_0.475ng/μl	0.6602905	M2_0.475ng/μl	0.49843484	M4_0.475ng/μl	0.465928137
F2_0.475ng/μl	0.4634119	F3_0.475ng/μl	0.5615138	M2_0.475ng/μl	0.49414507	M4_0.475ng/μl	0.465577245
<b>AVG</b>	<b>0.4955286</b>		<b>0.5915276</b>		<b>0.48977599</b>		<b>0.463749349</b>
<b>STDV</b>	<b>0.0340558</b>		<b>0.0597103</b>		<b>0.0114846</b>		<b>0.00347432</b>
<b>%CV</b>	<b>6.8726258</b>		<b>10.0942559</b>		<b>2.3448666</b>		<b>0.74918114</b>
F2_0.237ng/μl	0.2735329	F3_0.237ng/μl	0.2778296	M2_0.237ng/μl	0.23278247	M4_0.237ng/μl	0.238972008
F2_0.237ng/μl	0.2410643	F3_0.237ng/μl	0.2931946	M2_0.237ng/μl	0.21719648	M4_0.237ng/μl	0.201337412
F2_0.237ng/μl	0.2627469	F3_0.237ng/μl	0.3669961	M2_0.237ng/μl	0.20895158	M4_0.237ng/μl	0.20040752
<b>AVG</b>	<b>0.2591147</b>		<b>0.3126734</b>		<b>0.21964351</b>		<b>0.213572313</b>
<b>STDV</b>	<b>0.0165362</b>		<b>0.0476680</b>		<b>0.0121024</b>		<b>0.02200169</b>
<b>%CV</b>	<b>6.3818151</b>		<b>15.2452992</b>		<b>5.5100329</b>		<b>10.3017539</b>
F2_0.118ng/μl	0.0823981	F3_0.118ng/μl	0.0975576	M2_0.118ng/μl	0.04754364	M4_0.118ng/μl	0.112923652
F2_0.118ng/μl	0.0927059	F3_0.118ng/μl	0.1160402	M2_0.118ng/μl	0.04827278	M4_0.118ng/μl	0.099866278
F2_0.118ng/μl	0.0881587	F3_0.118ng/μl	0.1018040	M2_0.118ng/μl	0.0505398	M4_0.118ng/μl	0.123954065
<b>AVG</b>	<b>0.0877542</b>		<b>0.1051339</b>		<b>0.0487854</b>		<b>0.112247999</b>
<b>STDV</b>	<b>0.0051658</b>		<b>0.0096808</b>		<b>0.0015625</b>		<b>0.0120581</b>
<b>%CV</b>	<b>5.8866661</b>		<b>9.2080549</b>		<b>3.2027488</b>		<b>10.7423731</b>
F2_0.059ng/μl	0.0133791	F3_0.059ng/μl	0.0260898	M2_0.059ng/μl	0.01296957	M4_0.059ng/μl	0.024964811
F2_0.059ng/μl	0.0127685	F3_0.059ng/μl	0.0283383	M2_0.059ng/μl	0.01231305	M4_0.059ng/μl	0.028509557
F2_0.059ng/μl	0.0146727	F3_0.059ng/μl	0.0286605	M2_0.059ng/μl	0.01667418	M4_0.059ng/μl	0.028242713
<b>AVG</b>	<b>0.0136068</b>		<b>0.0276962</b>		<b>0.0139856</b>		<b>0.027239027</b>
<b>STDV</b>	<b>0.0009723</b>		<b>0.0014005</b>		<b>0.0023514</b>		<b>0.00197404</b>
<b>%CV</b>	<b>7.1455852</b>		<b>5.0565433</b>		<b>16.813017</b>		<b>7.24711178</b>
F2_0.0295ng/μl	0.0046887	F3_0.0295ng/μl	0.0029807	M2_0.0295ng/μl	0.0021066	M4_0.0295ng/μl	0.007926634
F2_0.0295ng/μl	0.0040113	F3_0.0295ng/μl	0.0033163	M2_0.0295ng/μl	0.00162966	M4_0.0295ng/μl	0.00705031
F2_0.0295ng/μl	0.0064391	F3_0.0295ng/μl	0.0036216	M2_0.0295ng/μl	0.0024548	M4_0.0295ng/μl	0.00833365
<b>AVG</b>	<b>0.0050464</b>		<b>0.0033062</b>		<b>0.00206369</b>		<b>0.007770198</b>
<b>STDV</b>	<b>0.0012528</b>		<b>0.0003205</b>		<b>0.0004142</b>		<b>0.00065582</b>
<b>%CV</b>	<b>24.825045</b>		<b>9.6949929</b>		<b>20.073008</b>		<b>8.44014778</b>
F2_0.0149ng/μl	0.001373	F3_0.0149ng/μl	0.0004757	M2_0.0149ng/μl	0.0008173	M4_0.0149ng/μl	0.003335109
F2_0.0149ng/μl	0.0014543	F3_0.0149ng/μl	0.0008819	M2_0.0149ng/μl	0.00107909	M4_0.0149ng/μl	0.002878756
F2_0.0149ng/μl	0.0014612	F3_0.0149ng/μl	0.0008894	M2_0.0149ng/μl	0.00052288	M4_0.0149ng/μl	0.003103724
<b>AVG</b>	<b>0.0014295</b>		<b>0.0007490</b>		<b>0.00080642</b>		<b>0.003105863</b>
<b>STDV</b>	<b>4.902E-05</b>		<b>0.0002367</b>		<b>0.0002783</b>		<b>0.00022818</b>
<b>%CV</b>	<b>3.4290844</b>		<b>31.6022534</b>		<b>34.505782</b>		<b>7.34687124</b>
F2_0.0075ng/μl	0.0003661	F3_0.0075ng/μl	0.0025684	M2_0.0075ng/μl	0.00043442	M4_0.0075ng/μl	0.000713128
F2_0.0075ng/μl	0.0003839	F3_0.0075ng/μl	0.0005468	M2_0.0075ng/μl	0.00062037	M4_0.0075ng/μl	0.001148099
F2_0.0075ng/μl	0.0005987	F3_0.0075ng/μl	0.0002546	M2_0.0075ng/μl	0.00055394	M4_0.0075ng/μl	0.001170428
<b>AVG</b>	<b>0.0004496</b>		<b>0.0011233</b>		<b>0.00053624</b>		<b>0.001010552</b>
<b>STDV</b>	<b>0.0001295</b>		<b>0.0012600</b>		<b>9.423E-05</b>		<b>0.00025782</b>
<b>%CV</b>	<b>28.798498</b>		<b>112.1733346</b>		<b>17.57158</b>		<b>25.512617</b>
F2_0.00385ng/μl	0.000329	F3_0.00385ng/μl		M2_0.00385ng/μl		M4_0.00385ng/μl	0.000834067
F2_0.00385ng/μl	0.0003573	F3_0.00385ng/μl		M2_0.00385ng/μl	0.00022634	M4_0.00385ng/μl	0.000919761
F2_0.00385ng/μl	0.0004083	F3_0.00385ng/μl	0.0001413	M2_0.00385ng/μl	0.00023895	M4_0.00385ng/μl	0.000507503
<b>AVG</b>	<b>0.0003649</b>		<b>0.0001413</b>		<b>0.00023264</b>		<b>0.000753777</b>
<b>STDV</b>	<b>4.02E-05</b>		<b>#DIV/0!</b>		<b>8.913E-06</b>		<b>0.00021754</b>
<b>%CV</b>	<b>11.017488</b>		<b>#DIV/0!</b>		<b>3.83125106</b>		<b>28.86014258</b>

**Table 2. Male [Y] DNA Sensitivity Series Quantitation Data performed using QuantStudio 5.**

Sample name_Dilution		[Y]	Sample name_Dilution		[Y]
M2_30ng/μl		32.19789886			
M2_30ng/μl		30.84238625			
M2_30ng/μl		33.91731644			
<b>AVG</b>		<b>32.31920052</b>			
<i>STDV</i>		<i>1.541049801</i>			
%CV		4.76821758			
M2_15ng/μl		14.11066532			
M2_15ng/μl		14.82552814			
M2_15ng/μl		14.82180977			
<b>AVG</b>		<b>14.58600108</b>			
<i>STDV</i>		<i>0.411657039</i>			
%CV		2.822274844			
M2_7.5ng/μl		7.091671944			
M2_7.5ng/μl		6.655923367			
M2_7.5ng/μl		7.673324585			
<b>AVG</b>		<b>7.140306632</b>			
<i>STDV</i>		<i>0.510441289</i>			
%CV		7.148730654			
M2_3.75ng/μl		3.736162186	M4_3.75ng/μl		2.899796963
M2_3.75ng/μl		3.893867493	M4_3.75ng/μl		3.397725821
M2_3.75ng/μl		3.784160614	M4_3.75ng/μl		3.396057606
<b>AVG</b>		<b>3.804730097</b>	<b>AVG</b>		<b>3.231193463</b>
<i>STDV</i>		<i>0.080839772</i>	<i>STDV</i>		<i>0.286999</i>
%CV		2.124717646	%CV		8.882136069
M2_1.9ng/μl		1.658705235	M4_1.9ng/μl		1.318773985
M2_1.9ng/μl		1.734183192	M4_1.9ng/μl		1.665314198
M2_1.9ng/μl		1.861801147	M4_1.9ng/μl		1.294127345
<b>AVG</b>		<b>1.751563191</b>	<b>AVG</b>		<b>1.426071843</b>
<i>STDV</i>		<i>0.102657371</i>	<i>STDV</i>		<i>0.207556119</i>
%CV		5.860900201	%CV		14.5543943
M2_0.95ng/μl		0.85132283	M4_0.95ng/μl		0.645673871
M2_0.95ng/μl		0.864166379	M4_0.95ng/μl		0.816659093
M2_0.95ng/μl		1.001016617	M4_0.95ng/μl		0.794672608
<b>AVG</b>		<b>0.905501942</b>	<b>AVG</b>		<b>0.752335191</b>
<i>STDV</i>		<i>0.082967036</i>	<i>STDV</i>		<i>0.093023272</i>
%CV		9.162546433	%CV		12.36460471

Sample name Dilution [Y]			Sample name Dilution [Y]		
	M2_0.475ng/μl	0.391085565		M4_0.475ng/μl	0.340132743
	M2_0.475ng/μl	0.439056247		M4_0.475ng/μl	0.335754186
	M2_0.475ng/μl	0.451706827		M4_0.475ng/μl	0.377735466
<b>AVG</b>		<b>0.42728288</b>	<b>AVG</b>		<b>0.351207465</b>
<b>STDV</b>		<b>0.031979579</b>	<b>STDV</b>		<b>0.023078</b>
<b>%CV</b>		<b>7.484404507</b>	<b>%CV</b>		<b>6.571044703</b>
	M2_0.237ng/μl	0.193048835		M4_0.237ng/μl	0.161480844
	M2_0.237ng/μl	0.186356053		M4_0.237ng/μl	0.139720589
	M2_0.237ng/μl	0.158788785		M4_0.237ng/μl	0.150114506
<b>AVG</b>		<b>0.179397891</b>	<b>AVG</b>		<b>0.150438646</b>
<b>STDV</b>		<b>0.018159013</b>	<b>STDV</b>		<b>0.010883748</b>
<b>%CV</b>		<b>10.12219991</b>	<b>%CV</b>		<b>7.23467572</b>
	M2_0.118ng/μl	0.038798582		M4_0.118ng/μl	0.081033379
	M2_0.118ng/μl	0.035740033		M4_0.118ng/μl	0.07502351
	M2_0.118ng/μl	0.040038511		M4_0.118ng/μl	0.083851568
<b>AVG</b>		<b>0.038192375</b>	<b>AVG</b>		<b>0.079969486</b>
<b>STDV</b>		<b>0.002212429</b>	<b>STDV</b>		<b>0.004509164</b>
<b>%CV</b>		<b>5.792856261</b>	<b>%CV</b>		<b>5.638605366</b>
	M2_0.059ng/μl	0.011260442		M4_0.059ng/μl	0.020082319
	M2_0.059ng/μl	0.008667623		M4_0.059ng/μl	0.019591097
	M2_0.059ng/μl	0.016726889		M4_0.059ng/μl	0.018477211
<b>AVG</b>		<b>0.012218318</b>	<b>AVG</b>		<b>0.019383542</b>
<b>STDV</b>		<b>0.004114133</b>	<b>STDV</b>		<b>0.000822437</b>
<b>%CV</b>		<b>33.67184209</b>	<b>%CV</b>		<b>4.24296475</b>
	M2_0.0295ng/μl	0.001462602		M4_0.0295ng/μl	0.006255205
	M2_0.0295ng/μl	0.001983923		M4_0.0295ng/μl	0.004502582
	M2_0.0295ng/μl	0.002325347		M4_0.0295ng/μl	0.006183818
<b>AVG</b>		<b>0.001923958</b>	<b>AVG</b>		<b>0.005647202</b>
<b>STDV</b>		<b>0.000434487</b>	<b>STDV</b>		<b>0.000991912</b>
<b>%CV</b>		<b>22.58299429</b>	<b>%CV</b>		<b>17.56467018</b>
	M2_0.0149ng/μl	0.000469602		M4_0.0149ng/μl	0.00254832
	M2_0.0149ng/μl	0.00064631		M4_0.0149ng/μl	0.001722858
	M2_0.0149ng/μl	0.000403919		M4_0.0149ng/μl	0.003210002
<b>AVG</b>		<b>0.00050661</b>	<b>AVG</b>		<b>0.002493727</b>
<b>STDV</b>		<b>0.000125361</b>	<b>STDV</b>		<b>0.000745073</b>
<b>%CV</b>		<b>24.74508977</b>	<b>%CV</b>		<b>29.87790482</b>
	M2_0.0075ng/μl	0.000436662		M4_0.0075ng/μl	0.000974402
	M2_0.0075ng/μl			M4_0.0075ng/μl	0.000698462
	M2_0.0075ng/μl			M4_0.0075ng/μl	0.000657371
<b>AVG</b>		<b>0.000436662</b>	<b>AVG</b>		<b>0.000776745</b>
<b>STDV</b>		<b>#DIV/0!</b>	<b>STDV</b>		<b>0.000172404</b>
<b>%CV</b>		<b>#DIV/0!</b>	<b>%CV</b>		<b>22.19574946</b>
	M2_0.00385ng/μl	0.000187332		M4_0.00385ng/μl	0.000277076
	M2_0.00385ng/μl	0.000275373		M4_0.00385ng/μl	0.000344701
	M2_0.00385ng/μl			M4_0.00385ng/μl	0.000405878
<b>AVG</b>		<b>0.000231352</b>	<b>AVG</b>		<b>0.000342552</b>
<b>STDV</b>		<b>6.22544E-05</b>	<b>STDV</b>		<b>6.4428E-05</b>
<b>%CV</b>		<b>26.90891817</b>	<b>%CV</b>		<b>18.80826349</b>

**Table 3. Male Autosomal DNA Sensitivity Series Quantitation Data performed using QuantStudio 5**

Sample name_Dilution	[AUTO]	Sample name_Dilution	[AUTO]	Sample name_Dilution	[AUTO]	Sample name_Dilution	[AUTO]
M1 0.95 ng/ul	0.9829	M3 0.95ng/ul	0.8251	M2 0.95 ng/ul	0.8527	M4 0.95 ng/ul	1.0201
M1 0.95 ng/ul	1.1604	M3 0.95ng/ul	0.8121	M2 0.95 ng/ul	0.9684	M4 0.95 ng/ul	1.1601
M1 0.95 ng/ul	1.0508	M3 0.95ng/ul	0.7696	M2 0.95 ng/ul	0.9934	M4 0.95 ng/ul	1.1257
<b>AVG</b>	<b>1.0647</b>	<b>AVG</b>	<b>0.8023</b>	<b>AVG</b>	<b>0.9382</b>	<b>AVG</b>	<b>1.1020</b>
<i>STDV</i>	<i>0.0896</i>	<i>STDV</i>	<i>0.0290</i>	<i>STDV</i>	<i>0.0751</i>	<i>STDV</i>	<i>0.0730</i>
% CV	8.4127	% CV	3.6193	% CV	8.0004	% CV	6.6202
M1 0.475 ng/ul	0.6016	M3 0.475 ng/ul	0.4180	M2 0.475 ng/ul	0.4374	M4 0.475 ng/ul	0.5373
M1 0.475 ng/ul	0.6229	M3 0.475 ng/ul	0.4362	M2 0.475 ng/ul	0.4678	M4 0.475 ng/ul	0.5689
M1 0.475 ng/ul	0.8654	M3 0.475 ng/ul	0.4066	M2 0.475 ng/ul	0.4529	M4 0.475 ng/ul	0.6148
<b>AVG</b>	<b>0.6966</b>	<b>AVG</b>	<b>0.4203</b>	<b>AVG</b>	<b>0.4527</b>	<b>AVG</b>	<b>0.5737</b>
<i>STDV</i>	<i>0.1465</i>	<i>STDV</i>	<i>0.0149</i>	<i>STDV</i>	<i>0.0152</i>	<i>STDV</i>	<i>0.0390</i>
% CV	21.0357	% CV	3.5491	% CV	3.3600	% CV	6.7987
M1 0.237 ng/ul	0.2429	M3 0.237 ng/ul	0.2065	M2 0.237 ng/ul	0.1941	M4 0.237 ng/ul	0.3070
M1 0.237 ng/ul	0.2654	M3 0.237 ng/ul	0.2264	M2 0.237 ng/ul	0.2278	M4 0.237 ng/ul	0.2633
M1 0.237 ng/ul	0.2530	M3 0.237 ng/ul	0.2240	M2 0.237 ng/ul	0.2152	M4 0.237 ng/ul	0.2603
<b>AVG</b>	<b>0.2538</b>	<b>AVG</b>	<b>0.2190</b>	<b>AVG</b>	<b>0.2123</b>	<b>AVG</b>	<b>0.2769</b>
<i>STDV</i>	<i>0.0113</i>	<i>STDV</i>	<i>0.0109</i>	<i>STDV</i>	<i>0.0170</i>	<i>STDV</i>	<i>0.0262</i>
% CV	4.4423	% CV	4.9642	% CV	8.0223	% CV	9.4479
M1 0.118 ng/ul	0.1339	M3 0.118ng/ul	0.1008	M2 0.118 ng/ul	0.1150	M4 0.118 ng/ul	0.1633
M1 0.118 ng/ul	0.1384	M3 0.118ng/ul	0.0931	M2 0.118 ng/ul	0.1245	M4 0.118 ng/ul	0.1495
M1 0.118 ng/ul	0.1391	M3 0.118ng/ul	0.0906	M2 0.118 ng/ul	0.1108	M4 0.118 ng/ul	0.1518
<b>AVG</b>	<b>0.1371</b>	<b>AVG</b>	<b>0.0948</b>	<b>AVG</b>	<b>0.1167</b>	<b>AVG</b>	<b>0.1549</b>
<i>STDV</i>	<i>0.0028</i>	<i>STDV</i>	<i>0.0053</i>	<i>STDV</i>	<i>0.0070</i>	<i>STDV</i>	<i>0.0074</i>
% CV	2.0543	% CV	5.5525	% CV	5.9974	% CV	4.7758
M1 0.059 ng/ul	0.0716	M3 0.059 ng/ul	0.0497	M2 0.059 ng/ul	0.0488	M4 0.059 ng/ul	0.0725
M1 0.059 ng/ul	0.0630	M3 0.059 ng/ul	0.0497	M2 0.059 ng/ul	0.0589	M4 0.059 ng/ul	0.0828
M1 0.059 ng/ul	0.0736	M3 0.059 ng/ul	0.0521	M2 0.059 ng/ul	0.0570	M4 0.059 ng/ul	0.0713
<b>AVG</b>	<b>0.0694</b>	<b>AVG</b>	<b>0.0505</b>	<b>AVG</b>	<b>0.0549</b>	<b>AVG</b>	<b>0.0756</b>
<i>STDV</i>	<i>0.0056</i>	<i>STDV</i>	<i>0.0014</i>	<i>STDV</i>	<i>0.0054</i>	<i>STDV</i>	<i>0.0063</i>
% CV	8.0887	% CV	2.6800	% CV	9.8215	% CV	8.3804
M1 0.0295 ng/ul	0.0284	M3 0.0295 ng/ul	0.0252	M2 0.0295 ng/ul	0.0245	M4 0.0295 ng/ul	0.0413
M1 0.0295 ng/ul	0.0288	M3 0.0295 ng/ul	0.0257	M2 0.0295 ng/ul	0.0285	M4 0.0295 ng/ul	0.0384
M1 0.0295 ng/ul	0.0291	M3 0.0295 ng/ul	0.0301	M2 0.0295 ng/ul	0.0308	M4 0.0295 ng/ul	0.0393
<b>AVG</b>	<b>0.0288</b>	<b>AVG</b>	<b>0.0270</b>	<b>AVG</b>	<b>0.0279</b>	<b>AVG</b>	<b>0.0397</b>
<i>STDV</i>	<i>0.0003</i>	<i>STDV</i>	<i>0.0027</i>	<i>STDV</i>	<i>0.0032</i>	<i>STDV</i>	<i>0.0015</i>
% CV	1.2049	% CV	9.8981	% CV	11.3668	% CV	3.6752
M1 0.0149 ng/ul	0.0156	M3 0.0149 ng/ul	0.0105	M2 0.0149 ng/ul	0.0129	M4 0.0149 ng/ul	0.0211
M1 0.0149 ng/ul	0.0216	M3 0.0149 ng/ul	0.0118	M2 0.0149 ng/ul	0.0149	M4 0.0149 ng/ul	0.0194
M1 0.0149 ng/ul	0.0205	M3 0.0149 ng/ul	0.0108	M2 0.0149 ng/ul	0.0124	M4 0.0149 ng/ul	0.0248
<b>AVG</b>	<b>0.0192</b>	<b>AVG</b>	<b>0.0111</b>	<b>AVG</b>	<b>0.0134</b>	<b>AVG</b>	<b>0.0218</b>
<i>STDV</i>	<i>0.0032</i>	<i>STDV</i>	<i>0.0007</i>	<i>STDV</i>	<i>0.0013</i>	<i>STDV</i>	<i>0.0028</i>
% CV	16.5531	% CV	6.1630	% CV	9.8180	% CV	12.7920
M1 0.0075 ng/ul	0.0088	M3 0.0075 ng/ul	0.0059	M2 0.0075 ng/ul	0.0057	M4 0.0075 ng/ul	0.0108
M1 0.0075 ng/ul	0.0066	M3 0.0075 ng/ul	0.0058	M2 0.0075 ng/ul	0.0078	M4 0.0075 ng/ul	0.0105
M1 0.0075 ng/ul	0.0098	M3 0.0075 ng/ul	0.0080	M2 0.0075 ng/ul	0.0071	M4 0.0075 ng/ul	0.0102
<b>AVG</b>	<b>0.0084</b>	<b>AVG</b>	<b>0.0066</b>	<b>AVG</b>	<b>0.0069</b>	<b>AVG</b>	<b>0.0105</b>
<i>STDV</i>	<i>0.0016</i>	<i>STDV</i>	<i>0.0012</i>	<i>STDV</i>	<i>0.0011</i>	<i>STDV</i>	<i>0.0003</i>
% CV	19.4931	% CV	18.6456	% CV	15.5313	% CV	2.9209
M1 0.00385 ng/ul	0.0034	M3 0.00385 ng/ul	0.0026	M2 0.00385 ng/ul	0.0037	M4 0.00385 ng/ul	0.0031
M1 0.00385 ng/ul	0.0041	M3 0.00385 ng/ul	0.0031	M2 0.00385 ng/ul	0.0041	M4 0.00385 ng/ul	0.0062
M1 0.00385 ng/ul	0.0047	M3 0.00385 ng/ul	0.0028	M2 0.00385 ng/ul	0.0033	M4 0.00385 ng/ul	0.0040
<b>AVG</b>	<b>0.0041</b>	<b>AVG</b>	<b>0.0028</b>	<b>AVG</b>	<b>0.0037</b>	<b>AVG</b>	<b>0.0045</b>
<i>STDV</i>	<i>0.0007</i>	<i>STDV</i>	<i>0.0003</i>	<i>STDV</i>	<i>0.0004</i>	<i>STDV</i>	<i>0.0016</i>
% CV	16.639	% CV	9.26077	% CV	11.0138	% CV	36.0642



**Table 4. Male [Y] DNA Sensitivity Series Quantitation Data performed using QuantStudio 5**

Sample name / Dilution	[Y]	Sample name / Dilution	[Y]	Sample name / Dilution	[Y]	Sample name / Dilution	[Y]
M1 0.95 ng/ul	0.8015	M3 0.95ng/ul	0.7295	M2 0.95 ng/ul	0.6870	M4 0.95 ng/ul	0.8792
M1 0.95 ng/ul	0.9159	M3 0.95ng/ul	0.6533	M2 0.95 ng/ul	0.8092	M4 0.95 ng/ul	0.9584
M1 0.95 ng/ul	0.8362	M3 0.95ng/ul	0.6428	M2 0.95 ng/ul	0.8225	M4 0.95 ng/ul	0.9627
<b>AVG</b>	<b>0.8512</b>	<b>AVG</b>	<b>0.6752</b>	<b>AVG</b>	<b>0.7729</b>	<b>AVG</b>	<b>0.9334</b>
<i>STDV</i>	<i>0.0586</i>	<i>STDV</i>	<i>0.0473</i>	<i>STDV</i>	<i>0.0747</i>	<i>STDV</i>	<i>0.0470</i>
%CV	6.8883	%CV	7.0097	%CV	9.6608	%CV	5.0337
M1 0.475 ng/ul	0.4687	M3 0.475 ng/ul	0.3446	M2 0.475 ng/ul	0.3546	M4 0.475 ng/ul	0.4414
M1 0.475 ng/ul	0.4850	M3 0.475 ng/ul	0.3593	M2 0.475 ng/ul	0.3853	M4 0.475 ng/ul	0.4709
M1 0.475 ng/ul	0.7131	M3 0.475 ng/ul	0.3404	M2 0.475 ng/ul	0.3696	M4 0.475 ng/ul	0.4900
<b>AVG</b>	<b>0.5556</b>	<b>AVG</b>	<b>0.3481</b>	<b>AVG</b>	<b>0.3698</b>	<b>AVG</b>	<b>0.4674</b>
<i>STDV</i>	<i>0.1366</i>	<i>STDV</i>	<i>0.0099</i>	<i>STDV</i>	<i>0.0153</i>	<i>STDV</i>	<i>0.0245</i>
%CV	24.5872	%CV	2.8581	%CV	4.1395	%CV	5.2403
M1 0.237 ng/ul	0.1887	M3 0.237 ng/ul	0.1773	M2 0.237 ng/ul	0.1571	M4 0.237 ng/ul	0.2301
M1 0.237 ng/ul	0.2031	M3 0.237 ng/ul	0.1976	M2 0.237 ng/ul	0.1781	M4 0.237 ng/ul	0.2215
M1 0.237 ng/ul	0.2040	M3 0.237 ng/ul	0.1842	M2 0.237 ng/ul	0.1804	M4 0.237 ng/ul	0.2019
<b>AVG</b>	<b>0.1986</b>	<b>AVG</b>	<b>0.1864</b>	<b>AVG</b>	<b>0.1718</b>	<b>AVG</b>	<b>0.2178</b>
<i>STDV</i>	<i>0.0086</i>	<i>STDV</i>	<i>0.0103</i>	<i>STDV</i>	<i>0.0128</i>	<i>STDV</i>	<i>0.0144</i>
%CV	4.3355	%CV	5.5408	%CV	7.4756	%CV	6.6317
M1 0.118 ng/ul	0.1042	M3 0.118ng/ul	0.0806	M2 0.118 ng/ul	0.0930	M4 0.118 ng/ul	0.1202
M1 0.118 ng/ul	0.1056	M3 0.118ng/ul	0.0753	M2 0.118 ng/ul	0.0936	M4 0.118 ng/ul	0.1186
M1 0.118 ng/ul	0.1053	M3 0.118ng/ul	0.0705	M2 0.118 ng/ul	0.0973	M4 0.118 ng/ul	0.1243
<b>AVG</b>	<b>0.1050</b>	<b>AVG</b>	<b>0.0755</b>	<b>AVG</b>	<b>0.0946</b>	<b>AVG</b>	<b>0.1211</b>
<i>STDV</i>	<i>0.0008</i>	<i>STDV</i>	<i>0.0050</i>	<i>STDV</i>	<i>0.0024</i>	<i>STDV</i>	<i>0.0029</i>
%CV	0.7157	%CV	6.6483	%CV	2.4906	%CV	2.4106
M1 0.059 ng/ul	0.0542	M3 0.059 ng/ul	0.0386	M2 0.059 ng/ul	0.0424	M4 0.059 ng/ul	0.0546
M1 0.059 ng/ul	0.0464	M3 0.059 ng/ul	0.0395	M2 0.059 ng/ul	0.0415	M4 0.059 ng/ul	0.0615
M1 0.059 ng/ul	0.0595	M3 0.059 ng/ul	0.0412	M2 0.059 ng/ul	0.0447	M4 0.059 ng/ul	0.0647
<b>AVG</b>	<b>0.0534</b>	<b>AVG</b>	<b>0.0398</b>	<b>AVG</b>	<b>0.0429</b>	<b>AVG</b>	<b>0.0603</b>
<i>STDV</i>	<i>0.0066</i>	<i>STDV</i>	<i>0.0013</i>	<i>STDV</i>	<i>0.0017</i>	<i>STDV</i>	<i>0.0052</i>
%CV	12.4243	%CV	3.2984	%CV	3.8689	%CV	8.6169
M1 0.0295 ng/ul	0.0204	M3 0.0295 ng/ul	0.0202	M2 0.0295 ng/ul	0.0211	M4 0.0295 ng/ul	0.0283
M1 0.0295 ng/ul	0.0240	M3 0.0295 ng/ul	0.0177	M2 0.0295 ng/ul	0.0209	M4 0.0295 ng/ul	0.0323
M1 0.0295 ng/ul	0.0250	M3 0.0295 ng/ul	0.0230	M2 0.0295 ng/ul	0.0289	M4 0.0295 ng/ul	0.0270
<b>AVG</b>	<b>0.0231</b>	<b>AVG</b>	<b>0.0203</b>	<b>AVG</b>	<b>0.0236</b>	<b>AVG</b>	<b>0.0292</b>
<i>STDV</i>	<i>0.0024</i>	<i>STDV</i>	<i>0.0026</i>	<i>STDV</i>	<i>0.0046</i>	<i>STDV</i>	<i>0.0028</i>
%CV	10.4319	%CV	13.0118	%CV	19.3342	%CV	9.4957
M1 0.0149 ng/ul	0.0111	M3 0.0149 ng/ul	0.0088	M2 0.0149 ng/ul	0.0100	M4 0.0149 ng/ul	0.0152
M1 0.0149 ng/ul	0.0142	M3 0.0149 ng/ul	0.0093	M2 0.0149 ng/ul	0.0109	M4 0.0149 ng/ul	0.0158
M1 0.0149 ng/ul	0.0156	M3 0.0149 ng/ul	0.0084	M2 0.0149 ng/ul	0.0106	M4 0.0149 ng/ul	0.0174
<b>AVG</b>	<b>0.0136</b>	<b>AVG</b>	<b>0.0088</b>	<b>AVG</b>	<b>0.0105</b>	<b>AVG</b>	<b>0.0161</b>
<i>STDV</i>	<i>0.0023</i>	<i>STDV</i>	<i>0.0004</i>	<i>STDV</i>	<i>0.0005</i>	<i>STDV</i>	<i>0.0011</i>
%CV	16.8056	%CV	4.8031	%CV	4.6346	%CV	6.9057
M1 0.0075 ng/ul	0.0067	M3 0.0075 ng/ul	0.0046	M2 0.0075 ng/ul	0.0054	M4 0.0075 ng/ul	0.0071
M1 0.0075 ng/ul	0.0062	M3 0.0075 ng/ul	0.0040	M2 0.0075 ng/ul	0.0051	M4 0.0075 ng/ul	0.0083
M1 0.0075 ng/ul	0.0066	M3 0.0075 ng/ul	0.0066	M2 0.0075 ng/ul	0.0050	M4 0.0075 ng/ul	0.0073
<b>AVG</b>	<b>0.0065</b>	<b>AVG</b>	<b>0.0050</b>	<b>AVG</b>	<b>0.0052</b>	<b>AVG</b>	<b>0.0076</b>
<i>STDV</i>	<i>0.0003</i>	<i>STDV</i>	<i>0.0013</i>	<i>STDV</i>	<i>0.0002</i>	<i>STDV</i>	<i>0.0006</i>
%CV	0.0004	%CV	0.0027	%CV	0.0004	%CV	0.0008
M1 0.00385 ng/ul	0.0026	M3 0.00385 ng/ul	0.0031	M2 0.00385 ng/ul	0.0027	M4 0.00385 ng/ul	0.0023
M1 0.00385 ng/ul	0.0031	M3 0.00385 ng/ul	0.0026	M2 0.00385 ng/ul	0.0023	M4 0.00385 ng/ul	0.0029
M1 0.00385 ng/ul	0.0040	M3 0.00385 ng/ul	0.0020	M2 0.00385 ng/ul	0.0026	M4 0.00385 ng/ul	0.0024
<b>AVG</b>	<b>0.0032</b>	<b>AVG</b>	<b>0.0026</b>	<b>AVG</b>	<b>0.0025</b>	<b>AVG</b>	<b>0.0025</b>
<i>STDV</i>	<i>0.0007</i>	<i>STDV</i>	<i>0.0005</i>	<i>STDV</i>	<i>0.0002</i>	<i>STDV</i>	<i>0.0003</i>
%CV	21.8222	%CV	20.5366	%CV	9.60421	%CV	13.6826

## Mixture studies

Mixture studies were performed using male:male and male:female mixtures. The first experiment compared the PQ results obtained using the QS5 instrument to the 7500 platform, which had been used previously to assess the performance of PQ verses Quantifiler™ Trio (Applied Biosystems, Foster City, CA).<sup>5</sup> Four mixture samples containing non-sperm fractions (nsp) and sperm fractions (sp) were utilized at the following semen dilutions: 1:100, 1:1000, 1:5000, and 1:5000. See table 5 below.

**Table 5. 7500 Real-Time PCR vs. QuantStudio 5 using male:male and male:female mixture samples.**

Sample Name	[AUTO]		[DEG]		[Y]		[Auto] / [D]	
	7500	Studio 5	7500	Studio 5	7500	Studio 5	7500	Studio 5
Male 1 + M5 100 nsp rep 1	2.4341	2.3221	0.3423	0.3543	1.5744	1.5327	7.11	6.55
Male 1 + M5 100 nsp rep 2	2.5983	2.0859	0.3450	0.3487	1.2483	1.3134	7.53	5.98
Male 1 + M5 100 nsp rep 3	2.4991	2.2056	0.3358	0.3846	1.2530	1.3635	7.44	5.74
AVG	<b>2.5105</b>	<b>2.2046</b>	AVG	<b>0.3410</b>	AVG	<b>1.3586</b>	AVG	<b>7.36</b>
STDV	0.0675	0.0964	STDV	0.0039	STDV	0.1526	STDV	0.18
%CV	2.6883	4.3737	%CV	1.1331	%CV	11.2359	%CV	2.46
Male 1 + M5 100 sp rep 1	0.5162	0.5336	0.1443	0.1521	0.3387	0.4724	3.58	3.51
Male 1 + M5 100 sp rep 2	0.4840	0.5513	0.1429	0.1419	0.3251	0.4330	3.39	3.88
Male 1 + M5 100 sp rep 3	0.5321	0.4922	0.1408	0.1410	0.3478	0.4045	3.78	3.49
AVG	<b>0.5108</b>	<b>0.5257</b>	AVG	<b>0.1427</b>	AVG	<b>0.3372</b>	AVG	<b>3.58</b>
STDV	0.0245	0.0304	STDV	0.0018	STDV	0.0114	STDV	0.20
%CV	4.7980	5.7738	%CV	1.2648	%CV	3.3892	%CV	5.49
Male 1 + F5 1000 nsp rep 1	15.2498	15.1912	2.9529	3.3333	0.0016	0.0042	5.16	4.56
Male 1 + F5 1000 nsp rep 2	16.6613	14.6193	3.1818	3.0541	0.0021	0.0023	5.24	4.79
Male 1 + F5 1000 nsp rep 3	15.5085	15.1760	3.2185	3.0425	0.0023	0.0017	4.82	4.99
AVG	<b>15.8065</b>	<b>14.9955</b>	AVG	<b>3.1177</b>	AVG	<b>0.0020</b>	AVG	<b>5.07</b>
STDV	0.7515	0.3258	STDV	0.1439	STDV	0.0003	STDV	0.22
%CV	4.7543	2.1730	%CV	4.6166	%CV	16.0928	%CV	4.40
Male 1 + F5 1000 sp rep 1	0.5819	0.5979	0.1043	0.0905	0.0413	0.0508	5.58	6.61
Male 1 + F5 1000 sp rep 2	0.5901	0.5719	0.0976	0.0933	0.0461	0.0516	6.05	6.13
Male 1 + F5 1000 sp rep 3	0.4792	0.6329	0.0925	0.1059	0.0445	0.0553	5.18	5.98
AVG	<b>0.5504</b>	<b>0.6009</b>	AVG	<b>0.0981</b>	AVG	<b>0.0440</b>	AVG	<b>5.60</b>
STDV	0.0618	0.0306	STDV	0.0059	STDV	0.0025	STDV	0.43
%CV	11.2281	5.0934	%CV	6.0353	%CV	5.5905	%CV	7.72
Male #3 + F4 5000 nsp rep 1	8.6956	10.4398	1.1592	1.6696		0.0011	7.50	6.25
Male #3 + F4 5000 nsp rep 2	9.5925	9.7615	1.2884	1.4318	0.0002	0.0004	7.45	6.82
Male #3 + F4 5000 nsp rep 3	10.2010	10.2058	1.1503	1.4880	0.0004	0.0003	8.87	6.86
AVG	<b>9.4964</b>	<b>10.1357</b>	AVG	<b>1.1993</b>	AVG	<b>0.0003</b>	AVG	<b>7.94</b>
STDV	0.7573	0.3445	STDV	0.0773	STDV	0.0001	STDV	0.81
%CV	7.9747	3.3993	%CV	6.4459	%CV	30.4570	%CV	10.15
Male #3 + F4 5000 sp rep 1	0.9719	0.9926	0.1281	0.1435	0.0057	0.0086	7.59	6.92
Male #3 + F4 5000 sp rep 2	1.0391	1.1382	0.1311	0.1368	0.0059	0.0084	7.93	8.32
Male #3 + F4 5000 sp rep 3	1.0890	1.0104	0.1492	0.1397	0.0041	0.0081	7.30	7.23
AVG	<b>1.0333</b>	<b>1.0471</b>	AVG	<b>0.1361</b>	AVG	<b>0.0052</b>	AVG	<b>7.60</b>
STDV	0.0588	0.0794	STDV	0.0114	STDV	0.0010	STDV	0.31
%CV	5.6868	7.5864	%CV	8.3867	%CV	18.8944	%CV	4.13
Male 1 + F6 50000 nsp rep 1	7.3639	5.6152	0.8269	0.7613			8.91	7.38
Male 1 + F6 50000 nsp rep 2	6.6426	6.3807	0.8488	1.0636			7.83	6.00
Male 1 + F6 50000 nsp rep 3	6.3996	6.0976	0.8120	1.0370		0.0003	7.88	5.88
AVG	<b>6.8020</b>	<b>6.0312</b>	AVG	<b>0.8292</b>	AVG	<b>0.0003</b>	AVG	<b>8.20</b>
STDV	0.5015	0.3870	STDV	0.0185	STDV		STDV	0.61
%CV	7.3733	6.4175	%CV	2.2354	%CV		%CV	7.41
Male 1 + F6 50000 sp rep 1	0.6553	0.6171	0.1180	0.1009	0.0003	0.0004	5.56	6.12
Male 1 + F6 50000 sp rep 2	0.5367	0.5607	0.1278	0.1071	0.0003	0.0006	4.20	5.24
Male 1 + F6 50000 sp rep 3	0.6742	0.5601	0.1457	0.0996	0.0002	0.0003	4.63	5.62
AVG	<b>0.6221</b>	<b>0.5793</b>	AVG	<b>0.1305</b>	AVG	<b>0.0002</b>	AVG	<b>4.79</b>
STDV	0.0746	0.0328	STDV	0.0141	STDV	0.0000	STDV	0.69
%CV	11.9846	5.6573	%CV	10.7798	%CV	18.2291	%CV	14.46

<sup>5</sup> Danielle Gibbes. Virginia Commonwealth University Masters' thesis. 2019.

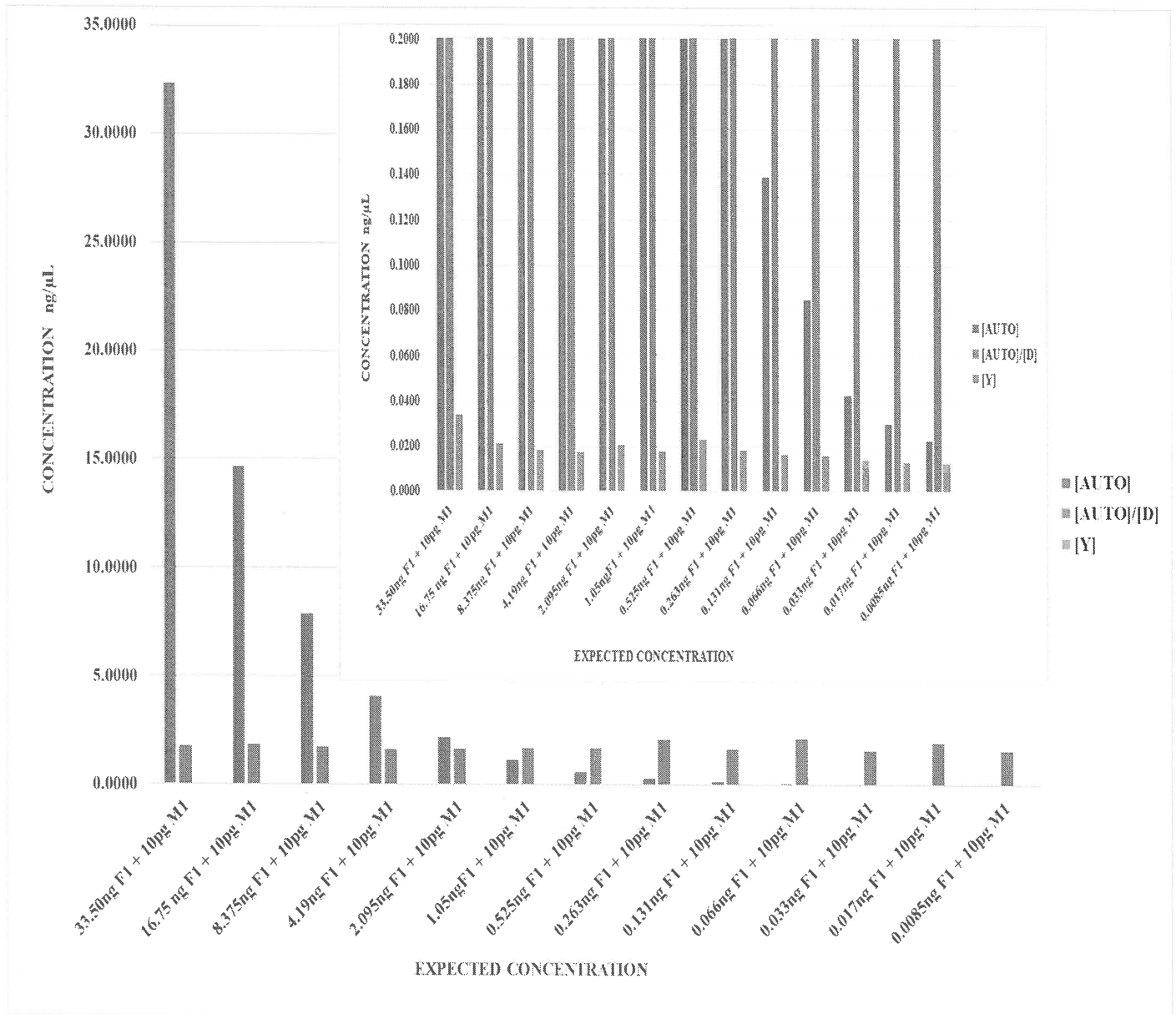
The results demonstrated that the precision and the range of PQ analysis on the QS5 is consistent with that of the 7500 System. Concentration estimates generated by the QS5 were similar to the expected DNA concentration based on the 7500 quantitation values and the DNA dilutions.

The next three experiments were designed to determine if the accuracy of the autosomal and male DNA concentrations was affected by the presence of an extreme excess of female DNA. For the mixture data shown in Table 6, male DNA (20 pg/ $\mu$ L) was mixed at equal volume (2.5 $\mu$ L each) with decreasing concentrations of female DNA (67.00 – 0.017 ng/ $\mu$ L). Both male and female samples were quantitated using PQ/QS5. The DNA quantity estimates were reasonably consistent and slightly below the expected value for autosomal and slightly above for Y-DNA concentrations.

**Table 6. Male (M1) – Female (F1) Mixtures - Male held constant with decreasing Female (DNA).**

Expected Concentration	[AUTO]	AUTO / D	[Y]
33.50 ng F1+ 10pg M1	32.3457	1.76	0.0336
16.75ng F1+ 10pg M1	14.6381	1.85	0.0210
8.375ng F1+ 10pg M1	7.8664	1.75	0.0182
4.19 ng F1+ 10pg M1	4.0809	1.64	0.0172
2.095 ng F1+ 10pg M1	2.1864	1.67	0.0206
1.05 ng F1+ 10pg M1	1.1569	1.70	0.0180
0.525 ng F1+ 10pg M1	0.6064	1.70	0.0230
0.263 ng F1+ 10pg M1	0.3159	2.12	0.0185
0.131 ng F1+ 10pg M1	0.1389	1.67	0.0164
0.066 ng F1+ 10pg M1	0.0845	2.13	0.0161
0.033 ng F1+ 10pg M1	0.0424	1.59	0.0140
0.017 ng F1+ 10pg M1	0.0298	1.94	0.0128
0.0085ng F1+ 10pg M1	0.0229	1.61	0.0126

The Auto/D degradation index is a measure of the degradation of the DNA samples. The degradation index consists of the autosomal target concentration estimate divided by the degradation target concentration estimate. The degradation target is 294 bp in size versus the 84 bp autosomal target and thus, due to the significant size differential of the amplicons, a ratio of the two provides an indication of the degree of the DNA degradation (degradation index). A value of  $>2$  indicates the DNA is degraded with the degree of degradation proportionate to the size of the value.<sup>2,5</sup> In Gibbes' study, the magnitude of the degradation index correlated well with the STR typing results (data not shown). The most degraded sample of a set of cadaver tissue samples provided an undetermined degradation index value since the target wasn't measurable. The corresponding STR profile only displayed results at seven loci (data not shown). Figure 1 displays the data in graph form including the Auto/Degradation ratio. The degradation index remained relatively constant for the different mixture dilutions.

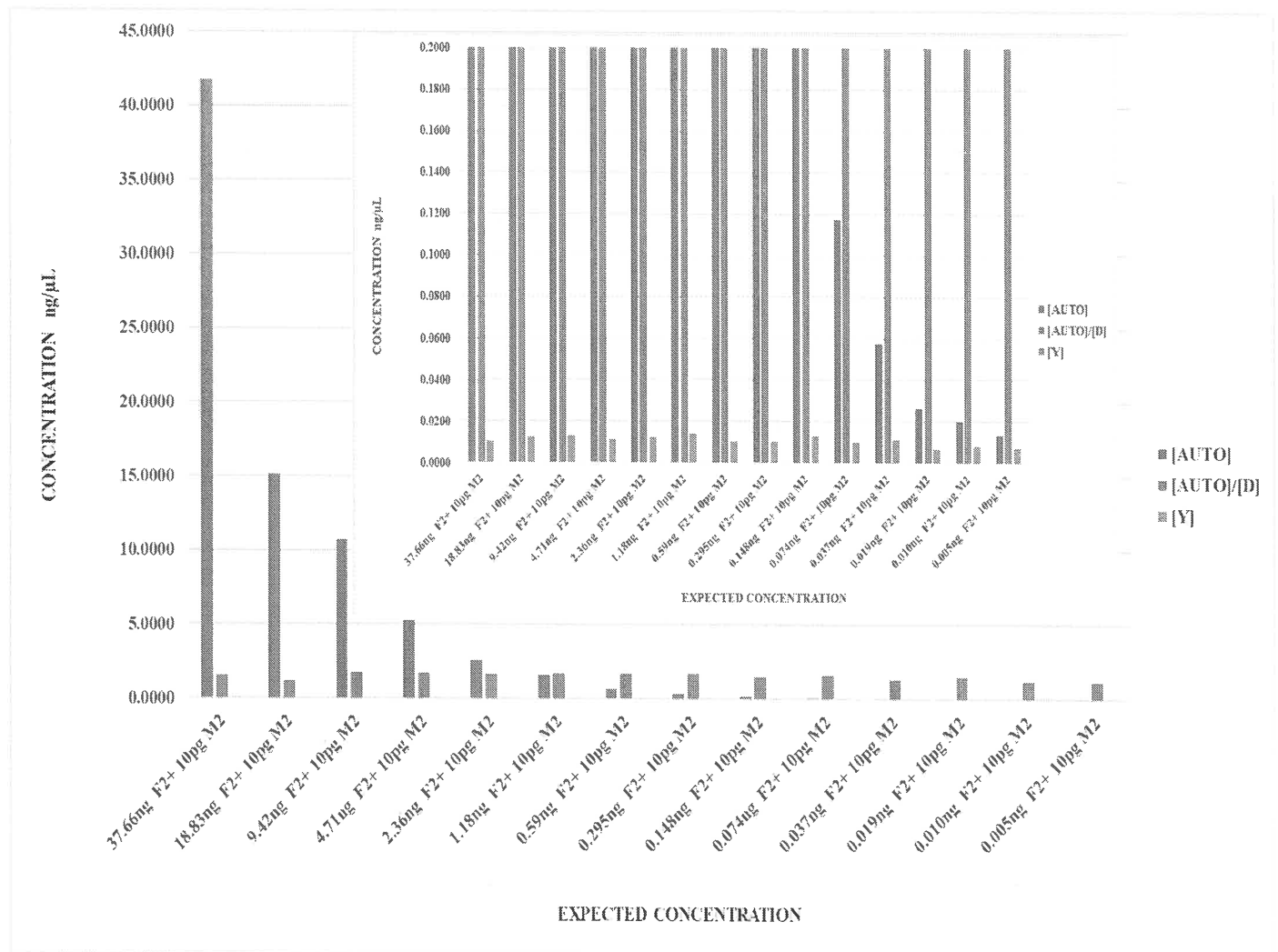


**Figure 1.** Male - female mixtures where male DNA (M1) was held constant with decreasing female DNA (F1). Inset shows the lower concentration samples for both female and male DNA.

For the mixture data shown in Table 7, male DNA (20 pg/ $\mu$ L) was mixed at equal volume (2.5 $\mu$ L) with decreasing concentrations of female DNA (75.32 – 0.010 ng/ $\mu$ L). Both male and female samples were quantitated using PQ/QS5. Quantitation values were relatively consistent and close to expected values for the Y-DNA, but also similar to the expected values for the autosomal DNA concentration.

**Table 7.** Male (M2) – female (F2) mixtures-male DNA was held constant with decreasing female DNA.

Expected Concentration	[AUTO]	[AUTO]/[D]	[Y]
37.66ng F2 + 10pg M2	41.7824	1.58	0.0108
18.83ng F2 + 10pg M2	15.1487	1.22	0.0130
9.42ng F2 + 10pg M2	10.7543	1.78	0.0132
4.71ng F2 + 10pg M2	5.2694	1.73	0.0114
2.36ng F2 + 10pg M2	2.6401	1.69	0.0125
1.18ng F2 + 10pg M2	1.6191	1.73	0.0140
0.59ng F2 + 10pg M2	0.7390	1.78	0.0108
0.295ng F2 + 10pg M2	0.3902	1.75	0.0108
0.148ng F2 + 10pg M2	0.2449	1.51	0.0132
0.074ng F2 + 10pg M2	0.1178	1.61	0.0102
0.037ng F2 + 10pg M2	0.0577	1.39	0.0114
0.019ng F2 + 10pg M2	0.0268	1.54	0.0070
0.010ng F2 + 10pg M2	0.0204	1.28	0.0084
0.005ng F2 + 10pg M2	0.0136	1.22	0.0078



**Figure 2.** Male - female mixtures where male DNA (M2) was held constant with decreasing female DNA (F2). Inset shows the lower concentration samples for both female and male DNA

The last male:female mixture series was conducted using samples M3 and F3. M3 male DNA (20 pg/μL) was mixed in equal volumes (2.5μL) with decreasing concentrations of F3 female DNA (65.44 – 0.017 ng/μL) (Table 8). The data demonstrated that both the male and autosomal DNA concentration estimates were also similar to expected values. However, at the lower concentrations, below the expected value of 0.065ng/μL for autosomal DNA and 0.010ng/μL for male DNA, the measured values were much lower than expected. Also, the male DNA estimate was far below the expected value at the 0.258 ng F3 +10 pg M3 dilution and below. These aberrant measures may be due to DNA sticking to the plastic tubes or pipetting error or a combination of the two.

**Table 8: Male (M3) - female (F3) mixtures where male DNA was held constant with decreasing female DNA.**

Well	Expected Concentration	[Auto]	[Auto]/ [D]	[Y]
A1	32.72 ng F3 +10 pg M3	32.0571	2.48	0.0146
B1	16.36 ng F3 +10 pg M3	15.0005	2.40	0.0094
C1	8.18 ng F3 +10 pg M3	6.9703	2.11	0.0085
D1	4.09 ng F3 +10 pg M3	3.8845	2.16	0.0074
E1	2.05 ng F3 +10 pg M3	2.0076	1.91	0.0091
F1	1.03 ng F3 +10 pg M3	1.0844	1.79	0.0102
G1	0.515 ng F3 +10 pg M3	0.5635	1.71	0.0146
H1	0.258 ng F3 +10 pg M3	0.3216	1.98	0.0091
A2	0.129 ng F3 +10 pg M3	0.1392	2.01	0.0086
B2	0.065 ng F3 +10 pg M3	0.0424	2.18	0.0031
C2	0.033 ng F3 +10 pg M3	0.0041	3.87	0.0010
D2	0.017 ng F3 +10 pg M3	0.0017	0.85	
E2	0.0085 ng F3 +10 pg M3	0.0004	Undetermined	0.0004

### ***Non-Probative Casework***

Seventeen non-probative casework samples were retrieved from the Normalization plate after being normalized for STR setup on the Biomek® NX<sup>P</sup> automation workstation. Samples were normalized based upon the Plexor™ HY autosomal DNA concentrations according to the VDFS procedures manual.<sup>6</sup> Following retrieval, the DNA extracts were manually quantitated using the PQ/QS5 system. While autosomal concentration could be compared to the expected normalized value (0.1 ng/μL), the Y-DNA target concentrations had to be extrapolated. The PQ/QS5 data for Y-chromosome DNA was manually calculated and then compared to the Y-chromosome PLX data (Tables 9 and 10).

Most of the autosomal concentrations were close to the expected 0.1 ng/μl however, five samples were significantly below the target. These samples included T19-5642.36CP at 0.0340 ng/μl, C19-7264.1.gun.tr at 0.0358 ng/μL, C19-7806.1.console.bld at 0.0357 ng/μL, C19-7872.1.seat.bld at 0.0456 ng/μL, and C19-7872.1.armrest.bld at 0.0452 ng/μL. There was also one sample significantly higher, sample C17-4441.3\_toothbrush.sal.re at 0.5384 ng/μL.

Similarly, the extrapolated Y-DNA quantitation values were close to expected with five being different by greater than a factor of two. These samples included: C19-7798.8723AD at an extrapolated

<sup>6</sup> Virginia Department of Forensic Science Forensic Biology Procedures Manual, PLEXOR® HY Quantitation of DNA.

concentration of 0.1542 ng/μL versus an observed concentration of 0.0525 ng/μL, C19-8376.2.shirt.wr at an extrapolated concentration of 0.0269 ng/μL versus an observed concentration of 0.1435 ng/μL, M19-1022.M2.bld at an extrapolated concentration of 0.0365 ng/μL versus an observed concentration of 0.1060 ng/μL, M19.1022.M3.bld at an extrapolated concentration of 0.1086 ng/μL versus an observed concentration of 0.0516 ng/μL, and C17-4441.3\_toothbrush.sal.re at an extrapolated concentration of 0.3833 ng/μL versus an observed concentration of 0.0992 ng/μL. The auto/Y DNA ratios deviated substantially between PQ and PLX for many samples. This may be due in part to the PQ use of two Y-targets. According to the published PQ literature, the use of the two Y-targets stabilizes the variability of the A/Y estimate and hence, possibly explains the difference.<sup>7</sup>

**Table 9: Normalized non-probative sample autosomal quantitation data.**

Well	Sample Name	PQ [Auto]	PQ [Auto]/[D]	PQ [Y]	PQ [Auto]/[Y]	PLX [Auto]/[Y]	PQ Predicted [Y]
A1	C19-7349.1MB	0.1491	1.32	0.1335	1.12	0.643836	0.0960
B1	C19-7349.2SW	0.0770	1.32	0.0609	1.26	1	
C1	C19-7789.8640TB	0.1173	1.13		Undetermined	1.545455	
D1	C19-7798.8723AD	0.1750	1.25	0.1542	1.14	0.3	0.0525
E1	T19-5642.36CP	0.0340	2.71		Undetermined	66666.67	
F1	C19-8020.9.JCB	0.1078	1.14	0.0868	1.24	0.644068	0.0695
A3	C19-7264.1.gun.tr	0.0358	2.51	0.0279	1.28	1.064516	0.0381
B3	C19-8376.2.shirt.wr	0.1016	1.74	0.0269	3.77	1.411765	0.1435
C3	C19-7806.1.console.bld	0.0357	1.41	0.0261	1.37	1.37931	0.0492
D3	C19-9193.1.dash.bld	0.0607	1.48	0.0477	1.27	0.612245	0.0372
E3	C19-7872.1.seat.bld	0.0456	1.78	0.0343	1.33	0.791667	0.0361
F3	C19-7872.1.armrest.bld	0.0452	1.53	0.0374	1.21	1	0.0452
G3	C19-6022.75.bandana.wr	0.0783	2.31	0.0486	1.61	1.2	0.0940
A5	M19-1022.M2.bld	0.0883	0.95	0.0365	2.42	1.2	0.1060
B5	M19.1022.M3.bld	0.1226	0.93	0.1086	1.13	0.421053	0.0516
C5	C19-8875.20_bld	0.0868	1.76	0.0519	1.67	0.75	0.0651
D5	C17-4441.3_toothbrush.sal.re	0.5384	1.71	0.3833	1.40	0.184211	0.0992

<sup>7</sup> Ewing MM, Thompson JM, McLaren RS, Purpero VM, Thomas KJ, Dobrowski PA, et al. Human DNA quantification and sample quality assessment: Developmental validation of the PowerQuant1 system. *Forensic Sci Int Genetics* (2016);23:166–177.



**Table 10: Normalization sample Y-chromosome DNA concentration expected versus observed values.**

Well	PQ [Y]	PQ Predicted [Y]
A1	0.1335	0.0960
B1	0.0609	0.0770
C1		
D1	0.1542	0.0525
E1		
F1	0.0868	0.0695
A3	0.0279	0.0381
B3	0.0269	0.1435
C3	0.0261	0.0492
D3	0.0477	0.0372
E3	0.0343	0.0361
F3	0.0374	0.0452
G3	0.0486	0.0940
A5	0.0365	0.1060
B5	0.1086	0.0516
C5	0.0519	0.0651
D5	0.3833	0.0992

## CONCLUSION

A series of experiments were conducted to evaluate the performance of the PowerQuant® DNA Quantification System in conjunction with the QuantStudio™ 5 Real-Time PCR Instrument. These data were compared to the Plexor™ HY System used in conjunction with the Stratagene MX3005P real-time PCR instrument. The results show that the precision of the PQ/QS5 system is similar to that of the PLX system for sensitivity and detection of the low template male DNA in a mixture with a large excess of female DNA.<sup>8</sup> However, the PQ/QS5 system appeared to demonstrate superior precision since the %CV was consistently lower and more predictable than what was measured for the PLX process. Nearly all quantitation measurements for the PQ/QS5 system with a %CV greater than 10% were for DNA concentrations lower than 0.059ng/μl.

The PQ/QS5 system displayed a sensitivity and accuracy similar to the Plexor/Strat system. While there were some differences between the expected autosomal and Y-DNA concentration estimates when non-probative sample were re-quantitated using the PQ/QS5 system, most values were similar and within a factor of two. The degradation index rose above 2.0 proportionately to the degree of degradation observed and thus can be useful for examiners when assessing the qualitative status of the DNA samples. Thus, the PowerQuant® DNA Quantification System, used in conjunction with the QuantStudio™ 5 Real-Time PCR Instrument, performed DNA quantitation estimates both accurately

<sup>8</sup> Virginia Department of Forensic Science: VALIDATION OF THE PLEXOR™ HY SYSTEM. 2008.

and reproducibly. Therefore, the PQ/QS5 system is reliable for estimation of both the autosomal and male DNA quantities in a forensic casework sample