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VIRGINIA DEPARTMENT OF FORENSIC SCIENCE**VALIDATION OF THE POWERQUANT® SYSTEM-COMPARISON WITH PLEXOR™ HY SYSTEM USING NON-PROBATIVE AND MOCK CASEWORK SAMPLES****Prepared in August, 2024**

This summary amends the previous summary (prepared in July, 2021) to include a more specific purpose statement.

PURPOSE

The purpose of this study was to determine if the STR amplification template quantities for autosomal and male DNA needed to be modified when DNA samples were quantitated using PowerQuant® versus Plexor HY™. This study compared the estimated autosomal and male DNA concentrations, measured using the PowerQuant® and Plexor® HY DNA Quantification Systems, for a variety of sample types and DNA quantities typically encountered in casework.

MATERIALS AND METHODS

A total of 22 DNA extracts representing a range of DNA concentrations was quantitated. Single source samples included blood (one of which was extremely degraded), buccal and a bone sample. Mixture samples included female buccal or vaginal samples mixed with different dilutions of seminal fluid on a variety of substrates and contaminants commonly encountered in casework.

Two NIST-traceable blood samples were manually extracted and purified using the DNA IQ™ System (DNA IQ; Promega Corp., Madison, WI), as described in the VDFS Procedures Manual.¹ The remaining samples were previously extracted and purified using DNA IQ on the Biomek® NX^P Automation Workstation (Beckman Coulter, Fullerton, CA), or organically extracted and purified/concentrated using a Microcon® DNA Fast Flow filter (MilliporeSigma, Burlington, MA), according to the Virginia Department of Forensic Science Procedures Manual.¹ The mock sexual assault samples were previously extracted using the same methods, with the exception that a differential extraction was performed, as described in the VDFS Procedures Manual.¹ DNA extracts were stored at 4°C or -20°C prior to quantitation. The extraction/purification method used for each sample, along with the sample type, substrate (if known) and any contaminant present, is shown in Table 1.

¹ Forensic Biology Procedures Manual. Extraction of DNA. Virginia Department of Forensic Science. Issued December 23, 2019.

Sample Description/Substrate	Sample Type	Extraction/Purification Method
C21-3xxx 1	bone	organic/Microcon
Sheet+Lubricant-Male2.1:50K+Female4	buccal/seminal fluid	DNA IQ
Sheet-Male2.1:50K+Female4	buccal/seminal fluid	DNA IQ
Carpet-Male2.1:10K+Female4	buccal/seminal fluid	DNA IQ
Underpants+Nonoxynol9-Male2.1:10K+Female4	buccal/seminal fluid	organic/Microcon
Sheet-Male2.1:10K+Female4	buccal/seminal fluid	DNA IQ
C21-5xxx 1 (degraded)	blood	organic/Microcon
R543024 IQ (Whatman card)	blood	DNA IQ
R543018 IQ (Whatman card)	blood	DNA IQ
Underpants-Male2.1:10K+Female4	buccal/seminal fluid	organic/Microcon
Denim+BabyOil-Male2.1:10K+Female4	buccal/seminal fluid	organic/Microcon
buc BTS IQ (cotton swab)	buccal	DNA IQ
Male3.1:10K+Female3 (cotton swab)	buccal/seminal fluid	DNA IQ
Male3.1:100K+Female3 (cotton swab)	buccal/seminal fluid	DNA IQ
Male2.1:75K+Female2 (cotton swab)	vaginal/seminal fluid	DNA IQ
Male1.1:75K+Female1 (cotton swab)	vaginal/seminal fluid	DNA IQ
buc MKV IQ (cotton swab)	buccal	DNA IQ
C21-5xxx 2	buccal	organic/Microcon
buc JG IQ (cotton swab)	buccal	DNA IQ
NIST.BS.A.070121 (Whatman card)	blood	DNA IQ
NIST.CB.A.070121 (Whatman card)	blood	DNA IQ
RB.NIST.070121	blank	DNA IQ

Table 1. Sample type and extraction/purification method of samples quantitated, along with substrate (if known). Mock sexual assault mixture sample non-sperm fractions were used for convenience, as sufficient DNA extract remained for quantitation.

Each DNA extract was quantified in triplicate in a single qPCR analysis for each quantitation assay. The Promega Plexor® HY System (Plexor HY) amplification and detection was performed utilizing the Stratagene Mx3005P instrument (Agilent Technologies, La Jolla, CA) and data analyzed with the Mx3005P MxPro QPCR Software (Agilent Technologies) and Plexor® Analysis Software, as described in the Virginia Department of Forensic Science Procedures Manual.² The Promega PowerQuant® assay (PowerQuant) was performed following the manufacturer's recommendations, with the exception that the PowerQuant Dilution Buffer (used to prepare the standards) was also used for the no-template control.³ Automated serial dilution of the male standard and reaction plate setup was performed using a robotic method developed specifically for the PowerQuant system on the Biomek® NX^P Automation Workstation. Amplification and detection were performed using the QuantStudio™ 5 Real-Time PCR instrument (Thermo Fisher Scientific, Waltham, MA), which was calibrated for the

² Forensic Biology Plexor® HY Quantitation of DNA, Procedures Manual. Virginia Department of Forensic Science. December 30, 2019.

³ PowerQuant® System Technical Manual. Promega. Revised 1/2020.

following dyes: FAM for the autosomal target (84-base-pair amplicon), CAL Fluor® Gold 540 for the male targets (81bp and 136bp), TMR for the internal positive control (IPC) (435bp), Quasar® 670 for the degradation target (294bp), and CXR for the passive reference dye. The raw data were collected with QuantStudio™ Design and Analysis Software (Thermo Fisher Scientific) ver. 1.5.1, and analyzed using the PowerQuant® Analysis Tool (Promega) ver. 1.0.0.0.

RESULTS

Consistent autosomal and male DNA concentration estimates were obtained among replicates in each assay. The individual male and autosomal DNA concentrations are listed in Tables 2 and 3, respectively, along with the averages for the three replicates.

Sample	[Y] (ng/μL)							
	PlexorHY			PLX Avg	PowerQuant			PQ Avg
C21-3xxx_1	0.005	0.005	0.006	0.005	0.0036	0.0034	0.0044	0.004
Sheet+Lubricant-Male2.1:50K+Female4	n/a	0.000	n/a	0.000				0.000
Sheet-Male2.1:50K+Female4	0.000	0.001	n/a	0.000				0.000
Carpet-Male2.1:10K+Female4	0.000	0.000	0.000	0.000				0.000
Underpants+Nonoxynol9-Male2.1:10K+Female4	0.001	0.002	0.002	0.002	0.0010	0.0017	0.0028	0.002
Sheet-Male2.1:10K+Female4	0.003	0.001	0.001	0.002	0.0012	0.0015	0.0016	0.001
C21-5xxx_1 (degraded)	0.012	0.020	0.015	0.016	0.0190	0.0197	0.0173	0.019
R543024_IQ (Whatman card)	0.600	0.750	0.740	0.697	0.6187	0.5463	0.5309	0.565
R543018_IQ (Whatman card)	1.400	1.600	1.700	1.567	0.8229	0.8832	0.8637	0.857
Underpants-Male2.1:10K+Female4	0.004	0.004	0.002	0.003	0.0027	0.0043	0.0044	0.004
Denim+BabyOil-Male2.1:10K+Female4	0.003	0.003	0.003	0.003	0.0053	0.0048	0.0034	0.004
buc_BTS_IQ (cotton swab)	3.000	2.600	2.700	2.767	0.9021	0.9295	0.9691	0.934
Male3.1:10K+Female3 (cotton swab)	n/a	0.000	0.000	0.000		0.0005		0.000
Male3.1:100K+Female3 (cotton swab)	n/a	n/a	n/a	0.000				0.000
Male2.1:75K+Female2 (cotton swab)	n/a	n/a	0.000	0.000				0.000
Male1.1:75K+Female1 (cotton swab)	0.002	0.000	0.001	0.001				0.000
buc_MKV_IQ (cotton swab)	21.000	16.000	24.000	20.333	5.3804	5.7863	5.6243	5.597
C21-5xxx_2	56.000	67.000	79.000	67.333	31.7940	31.7196	32.2539	31.922
buc_JG_IQ (cotton swab)	n/a	n/a	n/a	0.000				0.000
NIST.BS.A.070121 (Whatman card)	4.100	4.000	4.400	4.167	1.8939	1.8392	1.9860	1.906
NIST.CB.A.070121 (Whatman card)	4.000	4.600	4.100	4.233	2.2894	2.0954	2.4057	2.263
RB.NIST.070121	n/a	n/a	n/a	0.000				0.000

Table 2. Individual and average male DNA concentrations of extracts estimated using the Plexor HY and PowerQuant assays. No PowerQuant result displayed means that the DNA target was not detected.

Sample	[Auto] (ng/ μ L)							
	PlexorHY			PLX Avg	PowerQuant			PQ Avg
C21-3xxx_1	0.011	0.009	0.014	0.011	0.0042	0.0031	0.0032	0.004
Sheet+Lubricant-Male2.1:50K+Female4	0.230	0.180	0.240	0.217	0.0528	0.0648	0.0674	0.062
Sheet-Male2.1:50K+Female4	0.130	0.110	0.130	0.123	0.0539	0.0780	0.0743	0.069
Carpet-Male2.1:10K+Female4	1.100	0.980	1.100	1.060	0.2522	0.2790	0.2926	0.275
Underpants+Nonoxynol9-Male2.1:10K+Female4	0.960	1.200	0.880	1.013	0.4190	0.4951	0.4668	0.460
Sheet-Male2.1:10K+Female4	1.000	0.980	0.850	0.943	0.2986	0.3791	0.3610	0.346
C21-5xxx_1 (degraded)	0.110	0.086	0.083	0.093	0.0504	0.0684	0.0696	0.063
R543024 IQ (Whatman card)	0.310	0.300	0.310	0.307	0.2194	0.4940	0.4811	0.398
R543018 IQ (Whatman card)	0.370	0.520	0.230	0.373	0.6923	0.7424	0.7332	0.723
Underpants-Male2.1:10K+Female4	2.200	2.600	2.800	2.533	0.9997	0.9130	0.8925	0.935
Denim+BabyOil-Male2.1:10K+Female4	4.900	5.000	4.700	4.867	2.2548	2.1151	2.2592	2.210
buc BTS IQ (cotton swab)	1.200	1.400	1.600	1.400	1.1325	1.2147	1.3150	1.221
Male3.1:10K+Female3 (cotton swab)	54.000	64.000	64.000	60.667	9.2918	10.1762	9.9619	9.810
Male3.1:100K+Female3 (cotton swab)	37.000	36.000	30.000	34.333	6.4815	7.3440	7.1473	6.991
Male2.1:75K+Female2 (cotton swab)	35.000	37.000	32.000	34.667	13.2653	14.9627	14.3075	14.178
Male1.1:75K+Female1 (cotton swab)	27.000	27.000	23.000	25.667	17.8900	18.8955	18.4902	18.425
buc MKV IQ (cotton swab)	3.600	3.000	4.000	3.533	5.3629	6.2614	5.5645	5.730
C21-5xxx_2	22.000	26.000	37.000	28.333	31.6273	33.9882	31.2925	32.303
buc JG IQ (cotton swab)	25.000	24.000	23.000	24.000	9.0164	9.4129	9.6395	9.356
NIST.BS.A.070121 (Whatman card)	0.970	1.200	1.100	1.090	1.8733	1.8622	1.7807	1.839
NIST.CB.A.070121 (Whatman card)	1.700	1.600	1.700	1.667	1.9572	1.8731	1.9384	1.923
RB.NIST.070121	0.003	0.005	0.009	0.006				0.000

Table 3. Individual and average autosomal DNA concentrations of extracts estimated using the Plexor HY and PowerQuant assays. No PowerQuant result displayed means that the DNA target was not detected.

Most of the estimated average male DNA concentrations were similar for the Plexor HY and PowerQuant assays (Figure 1); however, four of the sample concentrations were estimated to be lower with PowerQuant by more than a factor of two. The correlation coefficient demonstrated high correlation (>0.9) between the two quantitation systems.

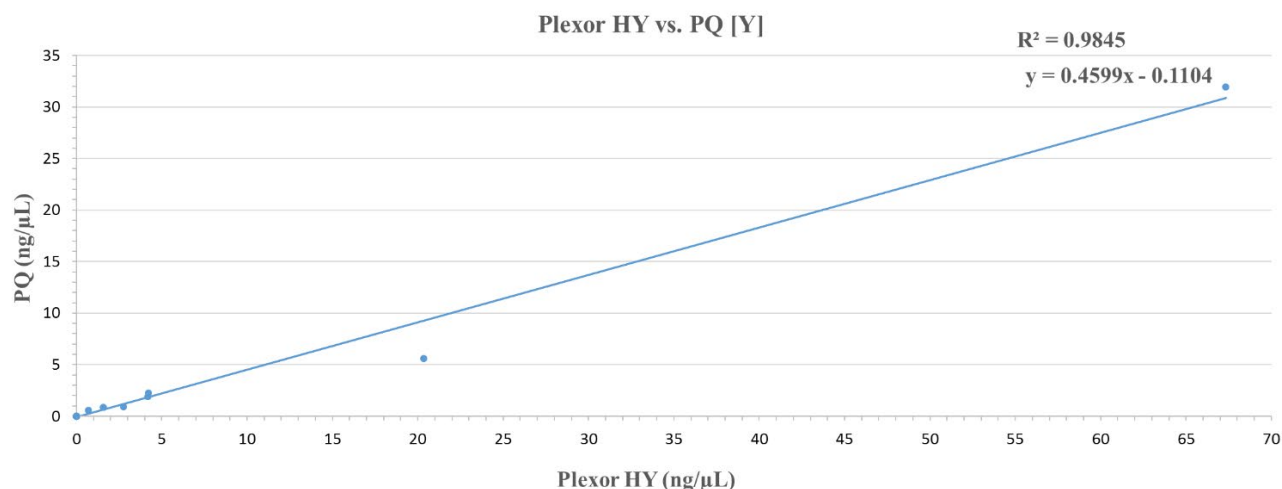


Figure 1. A comparison of average male DNA concentration estimates generated by Plexor HY versus PowerQuant (n=22).

The average autosomal DNA concentrations were not always highly correlated when the amount of DNA present was in the higher range (24-60ng/μL, as estimated by Plexor HY, Figure 2); however, the correlation coefficient was high (>0.8) between Plexor HY and PowerQuant estimates for both low and mid-range DNA concentrations (Figure 3). Eleven of the samples (approximately half) differed in the average quantity of DNA estimated by more than a factor of two. When a large difference was observed between average autosomal concentrations, the PowerQuant estimates were lower than the Plexor HY estimates.

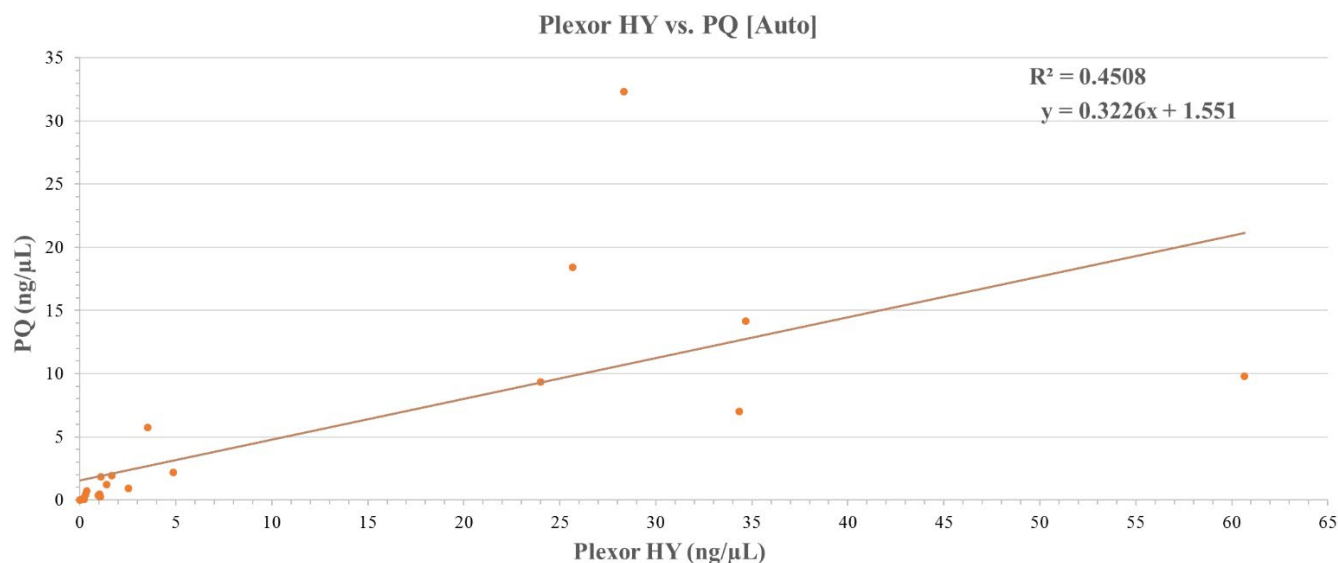


Figure 2. A comparison of all average autosomal DNA concentration estimates generated by Plexor HY versus PowerQuant (n=22).

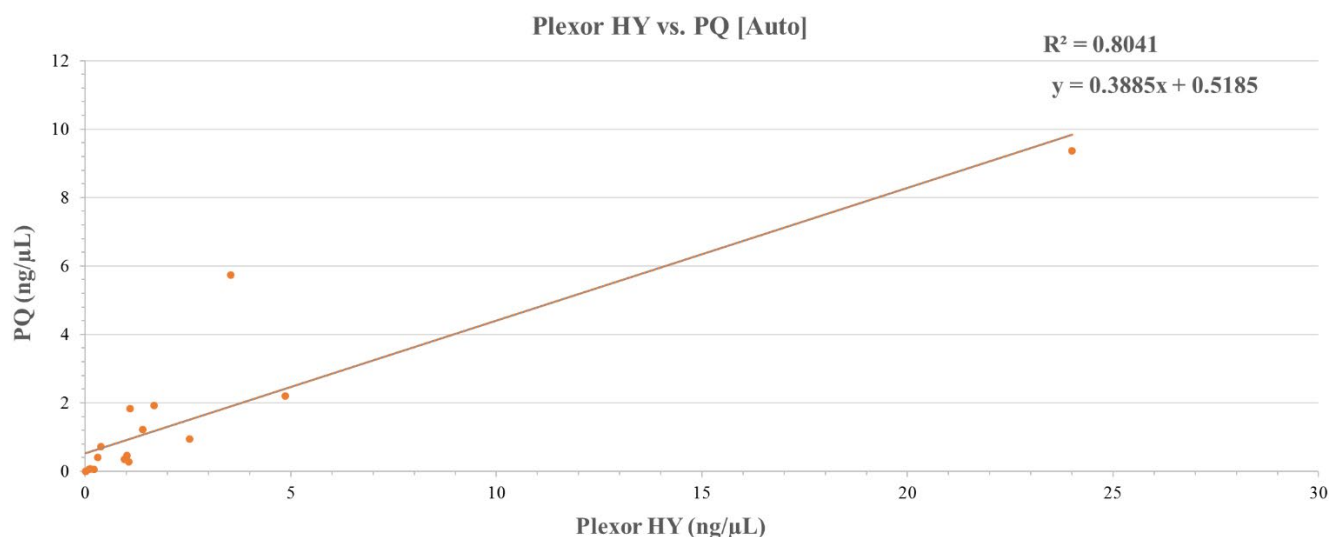


Figure 3. A comparison of average autosomal DNA concentration estimates generated by Plexor HY versus PowerQuant for low and mid-range concentrations. R^2 correlation increased when all samples of DNA concentration estimated at ≥ 25 ng/uL were removed (n=17).

If the DNA concentration of a sample measured is lower, this would have the same effect as increasing the amount of genomic template that is placed into the STR amplification cocktail. In practice, if artifacts are observed in the resulting DNA profile, a reduced injection time may be used, or the sample may be reinjected using less amplified DNA or using a dilution of the amplified DNA sample. It should be noted that, of the samples exhibiting a difference of greater than two-fold lower for PowerQuant versus Plexor HY, 1 of the 4 samples for the male target (C21-5xxx_2: 67.333 ng/μL, 31.922 ng/μL), and 3 of the 11 samples for the autosomal target (Male3.1:10K+Female3: 60.667 ng/μL, 9.810 ng/μL; Male3.1:100K+Female3: 34.333 ng/μL, 6.991 ng/μL; and Male2.1:75K+Female2: 34.667 ng/μL, 14.178 ng/μL), had an estimated concentration beyond the range of accuracy for the Plexor HY system (approx. 25 ng/μL)⁴. Most important is how well the quantitation values predict the amount of DNA needed in the amplification reaction to yield successful DNA typing results. The quantitation data generated for the validation of Casework Direct with the PowerQuant and QuantStudio system were compared with the STR typing outcomes for PowerPlex® Fusion (Fusion; Promega) and AmpFℓSTR® Yfiler® (Yfiler; Applied Biosystems, Foster City, CA). The completeness of male and female profiles and the allele RFUs obtained were evaluated to determine if the expected results were achieved when current amplification targets of 0.5ng input DNA for Fusion and 0.3ng input DNA for Yfiler were used, as described in the Virginia Department of Forensic Science Procedures Manuals.^{5,6} The STR typing results reflected the quantity of DNA amplified, as determined using PowerQuant.⁷

The PowerQuant system has been demonstrated to have improved precision over the Plexor HY system.⁸

CONCLUSION

Autosomal and male DNA concentrations, measured using the Plexor HY DNA and PowerQuant DNA Quantification Systems, were compared using a variety of non-probative and mock casework samples representing a range of DNA quantities. The two assays estimated similar male DNA concentrations for the majority of samples, and similar total human DNA concentrations for approximately half of the samples. For the remaining samples, the male or autosomal concentrations estimated with PowerQuant were at least two-fold lower than those measured with Plexor HY, particularly when the Plexor HY DNA concentration was estimated at approximately 25 ng/μL or greater. The PowerQuant quantitation results were more consistent with Plexor HY data for less concentrated (<25 ng/μL) samples. The Fusion and Yfiler DNA profile data generated during the validation of Casework Direct with the PowerQuant and QuantStudio system demonstrate that the PowerQuant method provides a reliable estimate for DNA typing of both the autosomal and male DNA quantities in a forensic sample. Additionally,

⁴ Virginia Department of Forensic Science Validation of the Plexor™ HY System. July, 2008.

⁵ Forensic Biology PowerPlex® Fusion Amplification and Long Term Storage, Procedures Manual. Virginia Department of Forensic Science. June 30, 2020.

⁶ Forensic Biology AmpFℓSTR® Yfiler® Amplification and Long Term Storage, Procedures Manual. Virginia Department of Forensic Science. June 30, 2020.

⁷ Virginia Department of Forensic Science. Validation of Casework Direct with PowerQuant. 2021.

⁸ 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 PowerQuant® system. *Forensic Sci Int Genetics* 2016;23:166-177.

if PowerQuant assesses the DNA concentration lower than Plexor HY, even in error, that result would be easily remedied by a variety of means such as: injection of the sample on the CE for a shorter time, loading less in the CE plate, and post-amplification dilution.