VALIDATION OF THE OHIO ATTORNEY GENERAL'S CFFS SCIENCE AUTOMATE *EXPRESS*™ ROBOT

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OHIO ATTORNEY GENERAL'S CENTER *for the* FUTURE *of* FORENSIC SCIENCE
AT BOWLING GREEN STATE UNIVERSITY

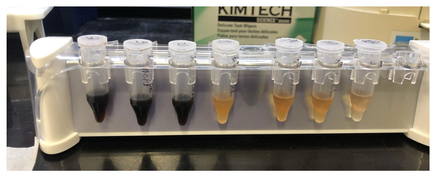
Introduction

The discovery of Deoxyribonucleic acid (DNA) extraction by Friedrich Miescher was pivotal for the scientific community as new research, technology, and methodology were developed as a result. As more information was discovered about DNA, these developments became specialized within different fields. Alec Jeffreys and his colleagues transformed the field of forensic science by utilizing the Restriction Fragment Length Polymorphism (RFLP) method and the Southern blotting technique to develop discriminating profiles using an organic extraction. Solid-phase extractions widened the range of abilities for DNA extraction as well as provided a method for automation in forensic laboratories. One instrument, the AutoMate™ *Express* robot, performs solid-phase DNA extractions with greater efficiency than manual methods. This validation of the AutoMate™ *Express* instrument adheres to the Quality Assurance Standards (QAS) developed by the Federal Bureau of Investigation (FBI) to maintain accuracy of methodology as well as uphold accreditation standards.

Methods

Two sets of samples underwent manual or robotic extraction methods. The first set had 26 samples of various biological fluids deposited from many different contributors. Four reagent blanks were analyzed with the samples through the entire process as well. The Ohio Attorney General's Center for the Future of Forensic Science Forensic Biology & DNA Standard Operating Procedures (SOP) Manual[14], derived from Applied Biosystems' protocols, was followed for the "Manual PrepFiler™" extraction procedure. The second set of 29 samples contained nearly the same samples as the first set, except for the addition of a few additional samples. These samples were prepared using the PrepFiler *Express*™ Forensic DNA Extraction Kit and run on the AutoMate *Express*™ instrument. The procedure performed followed the "AutoMate Express™ with PrepFiler *Express*™ Forensic DNA Extraction Kit" procedure outlined in the SOP Manual, based on the protocols developed by Applied Biosystems. Following extraction, samples were quantified on the QS5 Real-Time PCR System, amplified on the MiniAmp™ Thermal Cycler, and run on the 3500 Genetic Analyzer for capillary electrophoresis. These steps also followed the procedures outlined in the SOP Manual that were derived from Applied Biosystems protocols.

Between quantification and amplification steps, data was obtained to determine the amount of DNA in each sample using a standard curve with HID Real-Time PCR Analysis Software. This data also determined the amount of each sample that was amplified. After capillary electrophoresis, STR electropherograms were developed and peaks were analyzed for potential artifacts or contamination. STR interpretation procedure was followed according to the SOP Manual, which closely adhered to the FBI-QAS guidelines interpreted by SWGDAM in the interpretation guidelines document[15]. PDFs of each sample electropherogram were printed and data was exported into an Excel spreadsheet. Data was further organized and compared by peak heights for all samples.



Results

PARTIAL ELECTROPHEROGRAM FOR THE ROBOT "10R" SAMPLE

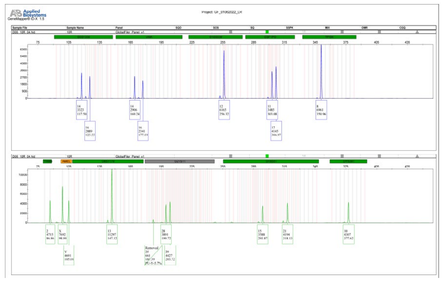


Figure 1. Blue and green dye channels are shown for the sample as well as the loci at each channel. Boxes with a diagonal line through them represent an artifact peak edited and removed from the data.

ROBOT EXTRACTION SAMPLE DATA

Description	Sample	D3S1358	vWA	D16S539	CSF1PO	TPOX	Yindel	AMEL	D8S1179	D21S11	D18S51	DYS391	D2S441	D19S433	TH01	FGA	D22S1045	D10S548	D1S1627	D7S820	SE33	D12S391	D2S1338
CO-Hair	1R	2788	2634.5	2121.5	2710	2123.5		5557.5	6023	3969	3526		4105.5	2807	3276.5	2597	4942.5	3877.5	4448.5	4870	3643.5	4179.5	5170.5	4387.5	3927.5
JRC Buccal	2R	7149	5794	4321	4454	3644.5	9158	10510	10204	6072	5871.5	6533	5877	5798.5	5736	4016.5	7546	6302	7919.5	5335	6950.5	9365	9274.5	6373	6891.5
CO Buccal	3R	5686.5	3039	3089	4277.5	3360.5		8073.5	7996.5	5036.5	4520		5584.5	5323.5	4804	4285	7091	5363.5	5407	4438	5357	7096	7005.5	5347.5	7276
Twin A Buccal on Flocked Swab	4R	11237.5	10196	10365.5	10616.5	8112		14530	20601	16869.5	13512.5		12526.5	13548.5	12773	10732	15499	11807	15107	15329.5	12765	17077.5	20653	14648.5	15571.5
Twin B Buccal on Flocked Swab	5R	4297.5	4144	3984	3545.5	2821		10107	8356.5	6875	5116.5		3321.5	2560	3317	2530	7385	6757	6929	5343.5	4650.5	9609.5	8716.5	7032.5	6071
Neat Male Saliva (Lot # M5632 (1S6025T3)	6R	2928.5	1969	1399	1104.5	1079	7482	5676.5	3813	3011.5	2166	5414	2914	3180	2270	1993.5	5381	3506.5	3061.5	2024.5	1442	5607.5	3563	1800.5	2074
Neat Male Saliva (Lot # M5632 (1S6025T3) with 0.1 g/mL Humic Acid	7R	4454	2837	2358	2201	1599.5	8191	8309	4672	3302.5	4264.5	5888	4246	4479.5	2890	3483.5	6688	4326	4443	4252.5	2835.5	7525.5	4537	2718	3626
Neat Male Blood HMW#82142	8R	5130	4560	5239.5	6425	5510.5	8224	10599.5	8035.5	5198	7918	5288	3289.5	3445.5	3388	4681	10713	9095	11055	7529.5	6868.5	9258.5	10531	4376.5	10864.5
Male Blood HMW#82614 (CM10) – 50 uL @ 220,000 cells	9R	2749	2806	3212	3210.5	2843.5	3991	6299	4691.5	5646	4093.5	3438	3313	2896	3453	2895	4527.5	3983	5674	4233	4513.5	4499.5	5504	3520	5691.5
Male Blood HMW#82614 (CM10) – 50 uL @ 220,000 cells	10R	3106	2623.5	3082.5	3815	3480.5	4715	6191.5	5648.5	4159	3891	4307	3842	3250.5	3797	4043	5418.5	4536	6500	3769.5	3457	3764	4751	2482	5307
Male Blood HMW#82614 (CM10) – 75 uL @ 330,000 cells	11R	3784.5	3899.5	3344.5	5373	3450.5	7091	9127	5788	5520	5864.5	5685	2539	2780	3961	3938	7546.5	7099.5	7837	5645	4842.5	6573	7530	3667	8448.5
Male Blood HMW#82614 (CM10) – 100 uL @ 440,000 cells	12R	2979.5	4476	3072.5	4736	2803.5	9618	9964	4900.5	5483	3941	3754	2063	2055	6783.5	4999.5	10862	8713.5	10306.5	7196	5342	6917	7078	3490.5	7249
Male Blood HMW#82995 (BM72) @ 220,000 cells on cotton	13R	2071.5	2454.5	2238.5	3778	2369.5	3188	4965	2234	3068.5	2497	2238	1478.5	1028	3015.5	2130.5	4533.5	3944.5	4154.5	1192	1583	4964	3452.5	1197	4037.5
Male Blood HMW#82995 (BM72) @ 330,000 cells on Flocked	14R	5404	6226.5	6752	9216	4788.5	9540	16254.5	11535	8126.5	8578	7063	4893.5	4846.5	10865	10848	10807	15393.5	13700	7724	6567	10539	9274.5	5917	11545
Male Blood HMW#82995 (BM72) @ 220,000 cells on dissolvable	15R	1514.5	1105.5	3132	3280.1	4421.5	8440	21139	8547.5	4446.5	3269.5	6637	1504	995	6814	17306	14818	14015.5	14873	2020.5	1118	15859	13555.5	2299	7586
NIST-6 (2391d) on FTA paper	16R	6804.5	5884	6000	5521.5	3649.5		13097.5	7449.5	6749	6408		4448.5	3684	4181.5	4090.5	8168	8431.5	9217.5	7092	6103.5	10004	11025	6558.5	8804
NIST Traceable Female Blood (L5241327)	17R	3125.5	6353	5150	7106.5	4338	9357	16074.5	5760.5	6005.5	4722.5	7986	3430.5	2205.5	6408	7025	11525	12193.5	12406.5	2969.5	4113.5	11193.5	10801	4228	8940
NIST Traceable Female Blood (L5241373)	18R	14139.5	16009	19136.5	12286	13045.5		13220	26450.5	24777.5	11853		24814	21870.5	14415.5	30889.5	14787.5	24958.5	26707.5	14035.5	12892	7338.5	22088.5	10147.5	27522
NIST Traceable Male Blood (L5241127) with 0.1 g/mL Humic Acid	19R	12965.5	20873.5	18379	13013	13914.5	28993	26477.5	26433	25861.5	24095	25560	15240.5	13207.5	12152.5	14278.5	31509.5	29160.5	28785	23439	16346.5	30843.5	31999.5	29831	25826.5
NIST Traceable Male Blood (L5241373) with 0.1 g/mL Humic Acid	20R	17562.5	26970.5	21681	15760	16269.5		34132.5	27995.5	28969	12012.5		30521.5	28920.5	11099	30308	15233.5	20402.5	21254	16050.5	11289.5	13854	20404	11545	27587
Neat Semen (Lot # 2771-01 IRH(9M51mL)	21R	3508.5	2914	2259	3915	1343	4188	3855	3156	4299.5	2835.5	3531	2052.5	1159	2062	2590.5	3737.5	4383	3513.5	3770.5	3319.5	5184	5342.5	2666	4720
1:10 Semen (Lot # 20-01-595 T6464)	22R	5177.5	4343	3998	4610.5	4357	6675	8362.5	6632	5508.5	6678.5	6262	3941	3540.5	5200	3861	5807	6184	8292	6885	5953.5	8402.5	8358.5	5938.5	9810
1:100 Semen (Lot # 2771-01 IRH(9M51mL)	23R	3172.5	3251.5	2469	2541	2335.5	5665	4079	4216	4172.5	3787.5	3467	2389	2663	3455.5	4222	4427	4553	4410	3428.5	3962	4593.5	3926.5	3606.5	5861.5
1:1000 Semen (Lot # 2771-01 IRH(9M51mL)	24R	4211.5	4027.5	3784.5	3568.5	3440	7500	7056.5	5905	4669.5	4936.5	5934	4442.5	3951.5	4420	4355	4719.5	4803.5	5920	5176	3757	8169	6496	4097.5	7937.5
1:10,000 Semen (Lot # 2771-01 IRH(9M51mL)	25R	872	891.5	766.5	1152.5	777	1420	1325	1475	1219.5	1334.5	1803	1525.5	1639.5	1621	1313.5	1331	1244	1009.5	1020	1068.5	702	1183.5	822	1304.5

Table 1. Example of data organization in Excel spreadsheet. Robot sample descriptions and corresponding names are located on the left of the table. Average peak heights for either heterozygous or homozygous alleles at each locus are reported horizontally for each sample. The peak heights correspond to both sample and STR locus.

ROBOT PEAK HEIGHT AVERAGES AND STANDARD DEVIATIONS FOR DYE CHANNELS

Avg. Heterozyougous Peak	Standard Devation
5552.30	4684.75
7963.46	6539.76
6235.61	6520.80
7977.03	6223.50
8558.72	6766.29

Table 2. Example of data organization for peak height averages and standard deviations per each dye channel. The dye channels are represented by their corresponding colors and were calculated using Excel formulas and the information provided from Table 1.

Discussion

Validations for new methodology and/or instrumentation help determine the efficiency and necessity for use in the laboratory. A method or instrument must be as good (preferably better) than the method/instrument that precedes it. In this validation of the AutoMate *Express*™, the instrument's efficiency was evaluated to determine whether the solid-phase extraction method was at least as efficient as the manual solid-phase extraction method. The efficiency can be determined by comparing data from both methods, performing the studies required by the FBI-QAS and interpreting data, as well as identifying limitations of the instrument. Comparison of peak heights among both robotic and manual samples afforded an indication of consistency among methods. The overall peak height average for all robotic samples at all loci was 7,258.76 RFUs. The overall peak height average for the manual samples was 7,268.36 RFUs. This indicates that the methods produced very similar results and that there was no large discrepancy between production of data among methods. The overall average PHR for the robot samples was 0.86 (86%) and 0.87 (87%) for the manual samples, revealing that the balance among alleles was consistent between methods. External data, using the same manual methodology as performed in this validation, found to have an overall PHR average of 0.88 (88%). Since this data was collected by a separate analyst, the results provided are therefore shown to be reproducible. This also signifies that data results were not highly influenced by the analyst that performed this validation. Overall, AutoMate *Express*™ methodology was shown to have the ability to produce data agreeable with the manual methodology.

Differing sample types and substrates provided information regarding parameters and optimization of the two methods. For instance, a male blood sample deposited on a dissolvable swab was analyzed using both the robot and manual procedures. The manual procedure was unable to produce results for the sample, while the robotic procedure could. The results were not as pristine as other samples, but they were interpretable regardless. Three other samples each with male blood substrate, differing only in concentration and cell amount, also differentiated method capabilities. The manual method determined the average peak height to increase with an increasing concentration as expected, except for the last sample. The robot method, however, was able to demonstrate the trend for all samples. Both methods calculated the PHR for each sample to be nearly the same. Data from a separate experiment using the AutoMate *Express*™ provided another sensitivity study that demonstrated similar results. Larger peak heights were observed for samples with greater starting concentration and PHRs were nearly the same among samples, contributing to the repeatability study for the instrument. Samples with different substrates were detectable among both methods with mostly equal efficiency; the peak heights for the same samples were very similar among both methods. Substrates, therefore, were not found to have a high impact on method optimization. Organic extraction data from another external experiment used the same NIST traceable male and female blood samples as used in this validation. The average peak height for the female sample on the robot was 18,268 RFUs and the male sample was 7224.35 RFUs. For the organic data, the female sample was 15,138.64 RFUs and the male sample was 7551.43 RFUs. The similarity of results indicates that the robot is able to produce agreeable data with the organic extraction.

The AutoMate *Express*™ instrument, as demonstrated by the results of this validation, is as efficient as both its organic and manual method precursors. The instrument has shorter operation time, lower potential for contamination, and greater efficiency for some samples as seen in the data comparison. It does, however, have a few limitations. Organic extractions and manual solid-phase extractions, for instance, may be better options for certain samples such as bones, teeth, and hair. The instrument is also unable to perform differential extractions, which the organic extraction is capable of. It would be prudent, therefore, for laboratories to maintain all three procedures since they each have better optimization for different samples. Regardless, the AutoMate *Express*™ instrument proves to be a practical option for use in conjunction with daily forensic laboratory work.

Conclusions

New DNA technology and methods are becoming more apparent as more discoveries and advancements are being made. It is important, therefore, to standardize and evaluate these methods as they are being utilized to draw conclusions impactful to individual lives. This is especially true in the forensic science field. The data produced by instrumentation and subsequent evaluations based on the methodology by analysts have the ability to indirectly impact the decision of an individual's freedom. This validation of the AutoMate *Express*™ instrument provides an example of the extent of evaluation recommended by the FBI that should be performed for new methodology/instrumentation. By providing studies and validation analysis, further forensic reports can be conducted ensuring high certainty of results with instrumentation/methodology used. Overall, while validations provide information on the efficiency of a method, they more importantly contribute to the greater cause of maintaining high ethical standards in the forensic science field.

1. Results of the validation found that the AutoMate™ Express instrument is a reliable, efficient method that is well suited for daily laboratory use.
2. Use of the AutoMate™ Express instrument would provide analysts with faster extraction of samples, as well as lower possibilities of contamination.
3. Overall, the AutoMate™ Express instrument proves to be a practical option for use in conjunction with daily laboratory work.

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