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TECHNICAL NOTE

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A Forensic Laboratory Tests the Berkeley Microfabricated Capillary Array Electrophoresis Device*

ABSTRACT: Miniaturization of capillary electrophoresis onto a microchip for forensic short tandem repeat analysis is the initial step in the process of producing a fully integrated and automated analysis system. A prototype of the Berkeley microfabricated capillary array electrophoresis device was installed at the Virginia Department of Forensic Science for testing. Instrument performance was verified by PowerPlex® 16 System profiling of single source, sensitivity series, mixture, and casework samples. Mock sexual assault samples were successfully analyzed using the PowerPlex® Y System. Resolution was assessed using the TH01, CSF1PO, TPOX, and Amelogenin loci and demonstrated to be comparable with commercial systems along with the instrument precision. Successful replacement of the Hjerten capillary coating method with a dynamic coating polymer was performed. The accurate and rapid typing of forensic samples demonstrates the successful technology transfer of this device into a practitioner laboratory and its potential for advancing high-throughput forensic typing.

KEYWORDS: forensic science, microfabricated capillary array electrophoresis, microchip, capillary electrophoresis, polyduramide, PowerPlex® 16, PowerPlex® Y

Technological advancements adopted by forensic science are driven by the need for improved efficiency. Whereas DNA profiling in the forensic arena has undergone dramatic changes in the past two decades from restriction fragment length polymorphism to PCR-based hybridization techniques to contemporary short tandem repeat (STR) typing (1–3), developments in technology continue to transpire that will ultimately produce dramatic changes in the manner in which DNA testing occurs, specifically in the detection, collection, and interpretation of amplified DNA products.

A fully integrated microchip system has the capacity to deliver greater benefits than increased speed of analysis. Integration of many of the steps of DNA typing onto microchip platform can make the entire process more automated, more robust, and require less user manipulation. Microfabricated capillary devices have been successfully integrated with on-chip thermocycling in the research laboratory venue (4–9). This seamless integration not only decreases the required reagent volumes and time consumed for the analysis process, but additionally reduces sample handling, which can eliminate the potential for sample mix-up and reduce the risk of contamination.

Increased speed of fragment separation is better realized using microfabricated capillary array electrophoresis (μCAE) devices than existing commercial capillary systems. Commercial capillary systems, such as the 16 capillary 3130*xl* Genetic Analyzer (Applied Biosystems [ABI], Foster City, CA) and also the 48 capillary 3730*xl* Genetic Analyzer (ABI) in use for forensic database samples can rapidly achieve fragment separation and detection. The μCAE device allows fast, high-quality DNA separation because of its capability to create a nanoliter sample plug that requires only short separation distances and fast dissipation of Joule heating under high voltages. Highly parallel DNA analyses of up to 384 samples have been previously demonstrated in <30 min on microfabricated devices (10–13). Moreover, advances in novel separation polymer synthesis will generate separation matrices with even greater resolving power that are ideal for microchip applications (14,15). Unique approaches to optimize capillary electrophoresis on microchip such as the integrated sample clean-up using affinity gel capture technology have the potential to enhance the performance of the capillary electrophoresis process in a hands-off, automated fashion (16–18). Additionally, portable capillary electrophoresis microchip instruments have been developed, thus providing us a glimpse into potential future directions in which DNA testing may evolve (5,19).

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Advances in microfabricated chip technologies are not limited to capillary electrophoresis. Microchips capable of DNA purification from samples that may be routinely encountered in a forensic laboratory have been successfully developed (20–22). Many research laboratories are pursuing a total automation system on microchip with "sample in, answer out" capabilities.

Technology developed in the basic research laboratory must be transitioned into the setting for which it is intended. That is the ultimate requirement for commercial instrumentation. Even though the microfabricated capillary array electrophoresis (μCAE) device is not a commercial system, we required its relocation as part of a collaborative effort between the laboratory of Dr. Richard Mathies at the University of California, Berkeley, the Virginia Department of Forensic Science (VDFS), and the Palm Beach County Sheriff's Office (PBSO). A prototype μCAE device was installed at VDFS for testing in a forensic laboratory by forensic scientists (Fig. 1). This study reports our efforts to master the operation of the μCAE device, test its performance using routine validation assessments (concordance, sensitivity, mixture analysis, and nonprobative sample typing), and evaluate improvements in its routine operation, such as the use of a dynamic coating polymer to coat the glass surface.

Materials and Methods

Sample Preparation

DNA samples for the concordance study were prepared from a total of 47 single-source DNA samples obtained from the research laboratory at VDFS, which included buccal swabs and dried blood cards. The 47 samples were extracted and purified manually using the DNA IQ™ System (Promega Corp., Madison, WI) according to the manufacturer's protocol with minor modifications for buccal cell samples and blood stains as described (23) and outlined in the VDFS procedure manual (24) or using an organic extraction procedure, followed by Micron YM-100 (Millipore Corp., Bedford, MA) clean-up as described (24). The DNA from either buccal swabs or tissue samples was purified for the sensitivity and mixture studies as defined (23) utilizing the robotic DNA IQ™ extraction procedure or using an organic extraction method as described above. Mock sexual assault samples were provided by Promega Corporation. Twelve samples were created by placing amounts of 1000 (1K), 10,000 (10K) or 50,000 (50K) spermatozoa on an epithelial swab (buccal or vaginal) and the swabs cut into equal halves for extraction. The mock sexual assault samples were extracted either with the semi-automated Differex™ method according to the vendor's protocol (25) or as described (23).

All samples were quantified with the AluQuant® Human DNA Quantitation System (Promega Corp.), utilizing a Biomek® 2000 Laboratory Automation Workstation (Beckman Coulter, Inc., Fullerton, CA) to set up the enzymatic reaction and the Luminoskan luminometer (Thermoelectron, West Palm Beach, FL) to detect the light signal produced. Samples were quantified with minor modifications from the manufacturer's protocol as outlined in the VDFS procedure manual and as described (24,26). Resulting concentration data were used to dilute the DNA extracts to a concentration of 0.15 ng/μL, for a total of 0.75 ng in the amplification reaction or as indicated.

The sensitivity series were created by placing the indicated quantity of diluted DNA into the amplification reaction. For mixture assays, two purified and quantified DNA samples were mixed together at differing ratios as indicated and placed into the PCR amplification reaction such that the total quantity of DNA amplified was 1 ng.

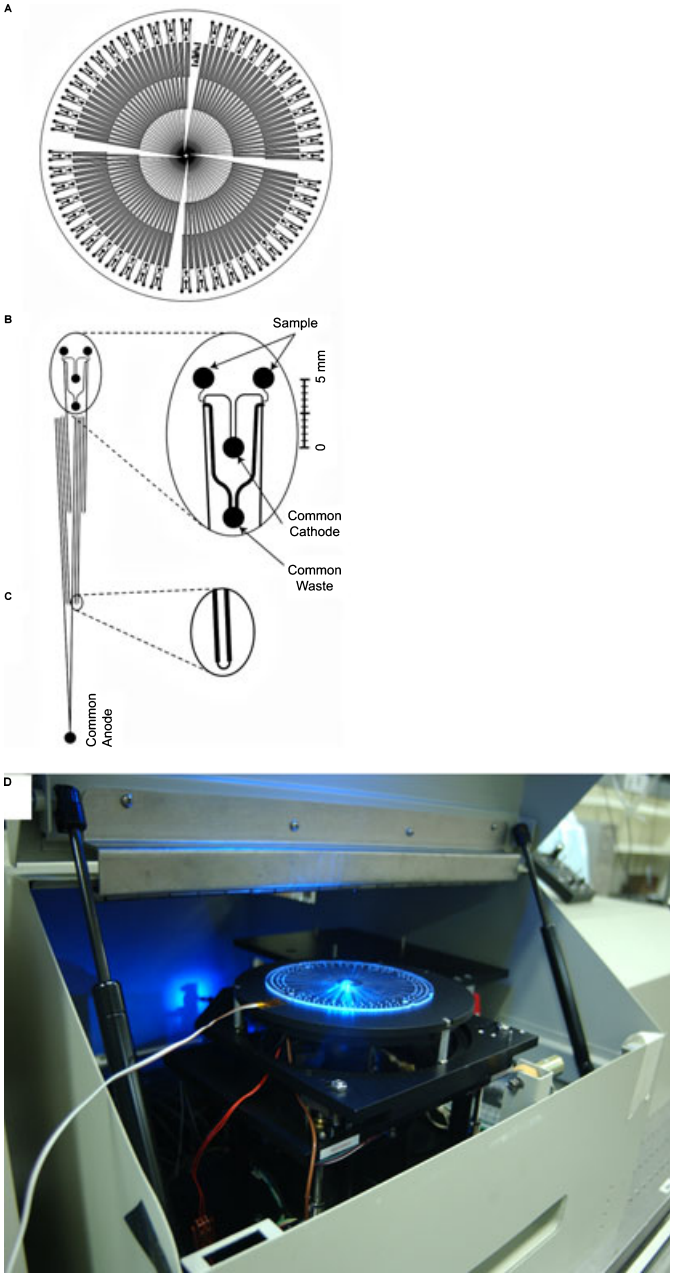

FIG. 1—(*A*) Design of the 96-channel microfabricated capillary array electrophoresis (μCAE) microchip used at VDFS. (*B*) Expanded view of channel doublet including two sample reservoirs and the common cathode and waste reservoirs. (*C*) Expanded view of a hyperturn within the channel. (*D*) Photograph of μCAE device prototype installed at VDFS. Figure modified from Paegel et al. (10).

Nonprobative Samples

Nineteen nonprobative samples from five different cases were provided by the PBSO laboratory. DNA extracts were quantified at PBSO with Quantifiler™ Human DNA Quantification Kit (ABI), utilizing a Biomek® 2000 Laboratory Automation Workstation (Beckman Coulter, Inc.) to set up the qPCR reaction and the ABI 7000 to detect fluorescent signal produced following the manufacturer's recommendations (27) and amplified using the PowerPlex® 16 System (Promega Corp.) following the manufacturer's recommendations (28). Samples were analyzed on both the μCAE device

and the ABI 310 and results were compared. The data produced from the μCAE device were sent to PBSO for comparison with previous DNA profiling results for the cases.

PCR Amplification

DNA samples were amplified using the PowerPlex® 16 System (PowerPlex® 16) or the PowerPlex® Y System (PowerPlex® Y), both manufactured by Promega Corp., multiplex STR amplification kits as described by the manufacturer (28,29); except for where indicated otherwise, PowerPlex® 16 amplified samples were amplified at half the manufacturer's recommended volume, as described (26). PCR amplification was completed in a GeneAmp System 9600 thermalcycler (ABI).

Separation and Detection

Where indicated, the STR amplicons were electrophoresed on both the ABI Prism® 310 Genetic Analyzer and the μCAE device. Preparation of samples for electrophoresis on the ABI 310 was as follows: 1 μL of each PCR product was added to a loading cocktail containing 24 μL Hi-Di™ formamide (ABI) and 1 μL Internal Lane Standard 600 (ILS600) (Promega Corp.). One allelic ladder sample was included for *c*. every 15 samples. Ladder was prepared in the same manner as PCR products with 1.0 μL PowerPlex® 16 or PowerPlex® Y Allelic Ladder Mix added to the injection cocktail. Samples were denatured for 3 min at 95°C and snap-cooled on ice prior to loading. Electrophoresis and data analysis were performed as recommended (28,29).

Preparation of the samples for electrophoresis on the μCAE was as follows: 1 μL of each PCR product was added to a loading cocktail containing 3 μL Hi-Di™ formamide, 3 μL sterile Type 1 H_2O, and either 1 or 0.75 μL ILS600 following the procedure used by Yeung et al. (13). Ladder was prepared by adding 2.0 μL PowerPlex® 16 Allelic Ladder Mix or PowerPlex® Y Allelic Ladder Mix (for PowerPlex® Y amplified samples) to a loading cocktail containing 2.5 μL Hi-Di™ formamide, 2.5 μL sterile Type 1 H_2O, and either 1 or 0.75 μL ILS600. Samples were denatured for 3 min at 95°C and snap-cooled on ice prior to loading.

Instrument Operation and Data Acquisition

For the ABI 310, the operation of the instrument followed the manufacturer's directions for use with the STR typing kits (28,29). The raw data were collected with ABI data collection software and analyzed using GeneScan and GenoTyper software, versions 3.1 and 2.5, respectively. Allele calls were performed using the PowerTyper™ 16 or PowerTyper™ Y Macros (Promega Corp.).

Operation of the μCAE device followed the procedures outlined by Yeung et al. (13). For μCAE chips coated using the modified Hjerten procedure (30), a fresh coating was applied every 2 weeks as recommended (S. Yeung, personal communication). For μCAE chips coated using the polyDuramide (pDuramide; 13) dynamic coating polymer, the procedure for coating was as follows: μCAE chips were first flushed with deionized sterile water (dH_2O) from the central anode to distribute fluid to all capillaries and sample wells, then the chips were filled with 1 M HCl and allowed to stand for 15 min, after which the chips were again flushed with dH_2O, followed by filling with the pDuramide and incubation for 15 min. After the pDuramide treatment, the chips were flushed with dH_2O, then dried and stored until use. The chips were employed for 5 days following coating after which a fresh coating was applied. To set up the chip, a polydimethylsiloxane (PDMS) elastomer ring was placed on top of the cathode and waste wells to create continuous buffer reservoirs. All 96 lanes of the chip were filled simultaneously through the central anode with MegaBACE™ Long Read Matrix linear polyacrylamide (GE Healthcare, Piscataway, NJ) using a high-pressure automatic gel loader. Gel was evacuated from the sample wells and replaced with 2.3 μL of each prepared sample or ladder loading cocktail. The μCAE chip was placed onto the instrument stage heated to 67°C prior to electrophoresis, an electrode array ring containing one electrode pin for each sample well was placed into the sample wells, and a smaller PDMS ring was placed around the central anode. The buffer reservoirs and central anode well were filled with 5× TTE (250 mM Tris, 250 mM N-Tris-hydroxymethyl methyl-3-aminopropanesulfonic acid [TAPS], 5 mM ethylene-diaminetetraacetate, pH 8.3) electrophoresis buffer. Sample injection occurred for 55–65 s at 170 V while grounding the sample wells and floating the cathode and the central anode wells. The parameters for electrophoresis in the capillary were: a grounded cathode, 2500 V applied to the anode, 200 V to the sample, and 200 V to the waste. Total run time was 28 min for the 96-channel chip to electrophorese PowerPlex® PCR products. Although the PowerPlex® Y amplicons were shorter and thus the total time could have been reduced, the same parameters were used for PowerPlex® Y product separation and detection. Following each run, the chip was placed into an automated high-pressure washer to flush the linear polyacrylamide out of the channels using deionized water. Raw data were collected with a custom LabView program (National Instruments, Austin, TX), the data files baseline corrected and annotated (LabView) appropriately for analysis using Genetic Profiler/Fragment Profiler software (GE Healthcare), then imported into Megabace Fragment Profiler v 1.2 software (GE Healthcare) for fragment sizing, color separation, and allele designation. Fragment Profiler v 1.2 does not perform peak smoothing of the data.

Appropriate peak filters and bin sets for PowerPlex® 16 and PowerPlex® Y were created using Fragment Profiler (v 1.2). Color separation matrices were also created using the Fragment Profiler software program. In lieu of commercially established threshold settings for peak heights, a signal to noise ratio (S/N) of 3:1 was applied.

Data Analysis for Resolution and Precision Studies

Only allelic ladder samples were used for both the precision and resolution calculations. For the ABI 310, two runs containing 15 and 13 allelic ladders, respectively, were completed for a total of 28 samples. Ladders were prepared for electrophoresis in the same manner as described above.

For the μCAE device, a total of 16 and 33 allelic ladder samples were successfully detected using the modified Hjerten and Poly-*N*-hydroxy-ethylacrylamide (pDuramide) coating procedures, respectively, through a series of four runs for both.

Precision Study

Sizing precision is defined as the ability to reproducibly estimate fragment sizes from run to run on any given instrument (31). Precision was calculated by averaging the standard deviation of size estimates across alleles at each locus. Within-run precision consisted of the standard deviation of size estimates for only those ladders contained within a single electrophoretic run, and between-run precision was calculated by combining data from all runs. All calculations for data from this study, as well as from previously generated data on the μCAE device were completed in Microsoft (MS) Excel.

Resolution Study

Resolution defined as the ratio of peak separation to the main peak width measures the ability of an instrument to separate components (31,32). A standard chromatographic equation to measure resolution relates the distance between two peaks to the widths of those peaks at half height (equation 1)

$$R = [2(\ln 2)]^{1/2}(\Delta X)/(W_{h1}+W_{h2}) \quad (1)$$

where ΔX is the peak to peak distance, W_{h1} is the width at half height of peak 1, and W_{h2} is the width at half height of peak 2 (32).

Two different measures of resolution related to this equation, R_b and resolution length (RSL) were calculated for this study as described by Buel et al. using both Amelogenin peaks, alleles 7 and 8 in TH01, alleles 9 and 10 in TPOX, and alleles 10 and 11 at CSF1PO (32).

R_b or base resolution gives the value of resolution in bases (equation 2)

$$R_b = \Delta M/R \quad (2)$$

where ΔM is the distance between two peaks in bases (32).

Resolution length is an alternate measurement which evaluates a single peak (equation 3)

$$\mathrm{RSL} = W_h/(\Delta X/\Delta M) \quad (3)$$

where W_h is the peak width at half height for the peak of interest, ΔX is the distance between this peak and an adjacent peak, and ΔM is the difference between the two peaks in bases (32).

A fourth resolution measurement, valley value (V_v), is an assessment of the resolution between peaks that differ in length by a single base (32), and was calculated for the 9.3 and 10 alleles of TH01 only (equation 4)

$$V_v = V/H \quad (4)$$

where V is equal to the height of the valley or the point where the two peaks merge and H is the peak height of the larger peak.

Measurements of peak widths, heights, and distances between peaks for data obtained in this study, as well as for data from the μCAE device previously generated at UC Berkeley, were obtained using calipers on printed electropherograms as described in Buel et al. (32). Distances between peaks in bases were obtained from the appropriate GenoTyper or Fragment Profiler software. All calculations were completed in MS Excel.

Results and Discussion

Concordance Study

Once successful operation of the μCAE had been established at VDFS, 47 single-source samples were amplified with PowerPlex® 16, electrophoresed, and analyzed. Allele calls were compared with the VDFS staff DNA index, typed using the PowerPlex® 16 BIO System as described (26,33) and PowerPlex® 16 profiles obtained from the same amplified samples analyzed using the ABI 310. All profiles obtained using the μCAE were identical with those generated using the ABI 310 and also with the VDFS staff DNA index (data not shown).

Use of Hjerten and pDuramide Coating Procedures

To prevent electro-osmotic flow as well as analyte adsorption, a Hjerten coating procedure of glass capillaries (30) is used in combination with a high sieving capacity linear polyacrylamide for efficient fragment separation and resolution (14,16,30). Drawbacks of the use of the Hjerten coating for microchip capillary electrophoresis are the propensity for capillary clogging and difficulties in applying the coating to all capillaries of the microcapillary array consistently. An alternate capillary coating procedure, pDuramide, a dynamic coating polymer, was evaluated for resolution, data quality, and ease of use (14,15). The use of the Long Read linear polymer acrylamide was still necessary as the separation polymer. Measurements for resolution performance, as well as sensitivity tests, both described in the Resolution, Precision, and Sensitivity studies, demonstrated that the pDuramide-coated chips performed nearly identically to the Hjerten-coated chips, with the advantage of greater ease-of-use. Thus, the pDuramide was used exclusively for microchip coating for all subsequent fragment separations. An added bonus was that the pDuramide coating process required less time (*c.* 30 min vs. *c.* 2 h).

Resolution and Precision Studies

Measures of resolution and precision were performed to assess the performance of the prototype μCAE instrument in combination with Fragment Profiler compared with a commercial capillary electrophoresis instrument employed by many forensic laboratories, the ABI 310. Precision calculations were performed using PowerPlex® 16 allelic ladder samples on both the ABI 310 and the μCAE, using modified Hjerten-coated microchips. As shown in Table 1, the ABI 310 displayed precision superior to the μCAE instrument used in the VDFS laboratory as well as the μCAE device data obtained from runs performed at the Mathies' laboratory. However, when the performance of the μCAE instrument is compared with reports for commercial multicapillary array instruments, rather than the single capillary ABI 310, the performances for the ABI 3100, 3700, and the MegaBACE 1000 are equivalent to the μCAE device. The sizing precision for the μCAE device using data produced at the Mathies' laboratory displayed a wider range than that produced at VDFS. That is probably because of the optimization of the instrument run parameters and software applications that were ongoing during the time frame in which the data were generated in the Mathies' laboratory as well as the less rigorous ambient temperature control compared with VDFS. Conditions utilized at VDFS varied little from those reported in the Yeung et al. paper (13).

Resolution measurements were derived as described in the Buel et al. report (32), providing a broad evaluation of capillary

TABLE 1—*Sizing precision.*

	Sizing Precision (SD)		
Instruments	Within-Run	Between-Run	±3 SD
ABI 310 (VDFS)	0.03–0.06 bp	0.03–0.06 bp	±0.18 bp
μCAE (VDFS)	0.02–0.23 bp	0.08–0.14 bp	±0.42 bp
μCAE (Mathies' lab data)	–	0.11–0.31 bp	±0.93 bp
ABI 377(37)	0.01–0.09 bp	–	–
ABI 377(38)	0.03–0.10 bp	–	–
ABI 310(31)	–	0.02–0.12 bp	±0.36 bp
ABI 310(37)	–	0.04–0.12 bp	–
ABI 310(39)	0.10 bp	0.20 bp	–
ABI 310(40)	0.01–0.13 bp	≤0.16 bp	–
ABI 3100(38)	–	0.03–0.17 bp	–
ABI 3700(38)	–	0.02–0.21 bp	–
FMBIO II(33)	–	–	±0.40–0.80 bp
MegaBACE 1000(38)	–	0.04–0.17 bp	–

VDFS, Virginia Department of Forensic Science; CAE, capillary array electrophoresis.

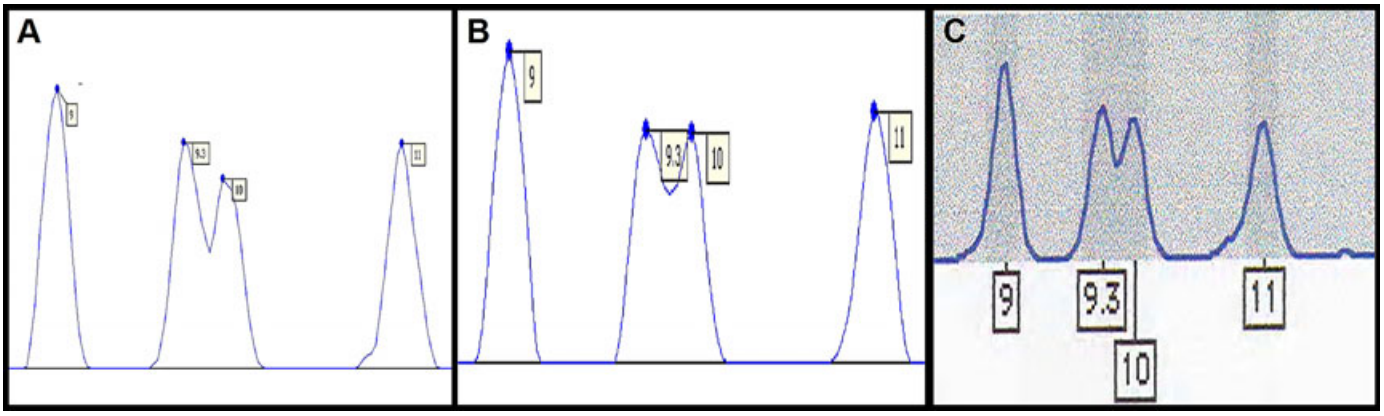

FIG. 2—*Separation of the 9.3/10 alleles of PowerPlex® 16 allelic ladder obtained on (A) microfabricated capillary array electrophoresis (μCAE) chip using the pDuramide, dynamic polymer coating. (B) μCAE chip using the modified Hjerten coating. (C) ABI 310 Genetic Analyzer.*

TABLE 2—*Measurements of resolution.*

Instruments	R_b	RSL	V_v
ABI 310 (VDFS)	1.15–1.72	0.67–0.99	0.64
μCAE (Mathies' lab data)	1.30–1.61	0.74–1.04	0.73
μCAE (VDFS) Hjerten coating	1.35–1.53	0.78–0.91	0.80
μCAE (VDFS) pDuramide coating	1.31–1.54	0.78–0.904	0.74
ABI 310(32)	1.04–1.64	0.61–0.96	0.51
ABI 310(40)	1.24–1.31	–	0.43–0.49
ABI 310(37)	–	–	~0.30
ABI 310(31)	1.13–1.49	–	–
μCAE(13)	1.3	0.76	–

VDFS, Virginia Department of Forensic Science; CAE, capillary array electrophoresis; RSL, resolution length; R_b, base resolution; V_v, valley value.

electrophoresis resolution. Larger molecular weight loci, such as CSF1PO as well as the smallest molecular weight loci, Amelogenin were evaluated using PowerPlex® 16 allelic ladder samples. As seen in Fig. 2 and Table 2, the base resolution (R_b), RSL, and the valley value (V_v) are similar to those reported by various sources for the ABI 310 and those measured by Yeung et al. (13) for the μCAE device using the TH01 locus. Values were also calculated by VDFS for the μCAE device using data generated in the Mathies' laboratory using loci (CSF1PO, TPOX, and Amelogenin) in addition to TH01. This was performed to compare it with the μCAE device operation at VDFS utilizing all of the same loci as Buel et al. for direct comparison. In addition, VDFS calculated values from μCAE device runs performed at VDFS using microchips coated with the modified Hjerten procedure as well as the pDuramide dynamic polymer coating. Values produced from data generated at VDFS for microchips coated using the two coatings were nearly identical and were similar to those produced for the μCAE device operated in the Mathies' laboratory. Moreover, peak morphology and the separation between the 9.3 and 10 alleles of the TH01 allelic ladder were virtually identical between the two different microchip coating procedures and were very similar to that produced by the ABI 310 (Fig. 2). Furthermore, the pDuramide-coated μCAE microchips demonstrated a significantly greater number of open, unclogged capillaries than the modified Hjerten-coated microchips; an average of *c.* 20% more open capillaries were obtained (n = 7 runs using Hjerten coating for a total of 672 capillaries; n = 8 runs using pDuramide for a total of 768 capillaries; data not shown).

TABLE 3—*Sensitivity data.*

	FGA	TPOX	D8	vWA	Amel	P.E.	D18	D21	TH01	D3	P.D.	CSF	D16	D7	D13	D5
VDFS DNA Sample—pDuramide Coating																
2 ng	22, 24	8, 11	10, 13	18, 20	X, Y	5, 13	11, 15	29, 30	9	15	10, 11	10, 11	10, 11	11	10, 11	11, 13
1 ng	22, 24	8, 11	10, 13	18, 20	X, Y	5, 13	11, 15	29, 30	9	15	10, 11	10, 11	10, 11	11	10, 11	11, 13
0.5 ng	22, 24	8, 11	10, 13	18, 20	X, Y	5, 13	11, 15	29, 30	9	15	10, 11	10, 11	10, 11	11	10, 11	11, 13
0.25 ng	22, 24	8, 11	10, 13	18, 20	X, Y	5, 13	11, 15	29, 30	9	15	10, 11	10, 11	10, 11	11	10, 11	11, 13
0.125 ng	22, 24	8, 11	10, 13	18, 20	X, Y	5, 13	11, 15	29, 30	9	15	10, 11	10	10, 11	11	10, 11	11, 13
0.062 ng	–	–	–	–	X, Y	–	11	–	9	15	–	–	–	–	–	
0.031 ng	–	8, 11	–	18	X, Y	5	15	–	9	15	–	–	–	–	–	–
0.015 ng	–	–	–	–	Y	–	–	–	9	15	–	–	–	–	–	–
NIST Sample—Modified Hjerten Coating																
10 ng	19, 23	8	14	17, 18	X, Y	5, 16	14, 16	31.2, 33.2	6, 7	16	11, 14	11, 12	9, 11	9, 11	8, 12	11, 12
5 ng	19, 23	8	14	17, 18	X, Y	5, 16	14, 16	31.2, 33.2	6, 7	16	11, 14	11, 12	9, 11	9, 11	8, 12	11, 12
2.5 ng	19, 23	8	14	17, 18	X, Y	5, 16	14, 16	31.2, 33.2	6, 7	16	11, 14	11, 12	9, 11	9, 11	8, 12	11, 12
1.25 ng*	19, 23	8	14	17, 18	X, Y	5, 16	14, 16	31.2, 33.2	6, 7	16	11, 14	11, 12	9, 11	9, 11	8, 12	11, 12
0.62 ng	19, 23	8	14	17, 18	X, Y	5, 16	14, 16	31.2, 33.2	6, 7	16	11, 14	11, 12	9, 11	9, 11	8, 12	11, 12
0.31 ng	19, 23	8	14	17, 18	X, Y	5, 16	14, 16	31.2, 33.2	6, 7	16	11, 14	11, 12	9, 11	9, 11	8, 12	11, 12
0.15 ng	19	8	14	17, 18	X, Y	5	14, 16	31.2, 33.2	7	16	14	12	9	11	–	–

VDFS, Virginia Department of Forensic Science; CAE, capillary array electrophoresis; Amel, Amelogenin; D8, D8S1179; P.E., Penta E; D18, D18S51; D21, D21S11; D3, D3S1358; P.D., Penta D; D16, D16S539; D7, D7S820; D13, D13S317; D5, D5S818.

*Data for sample obtained from a μCAE device run performed on a different day.

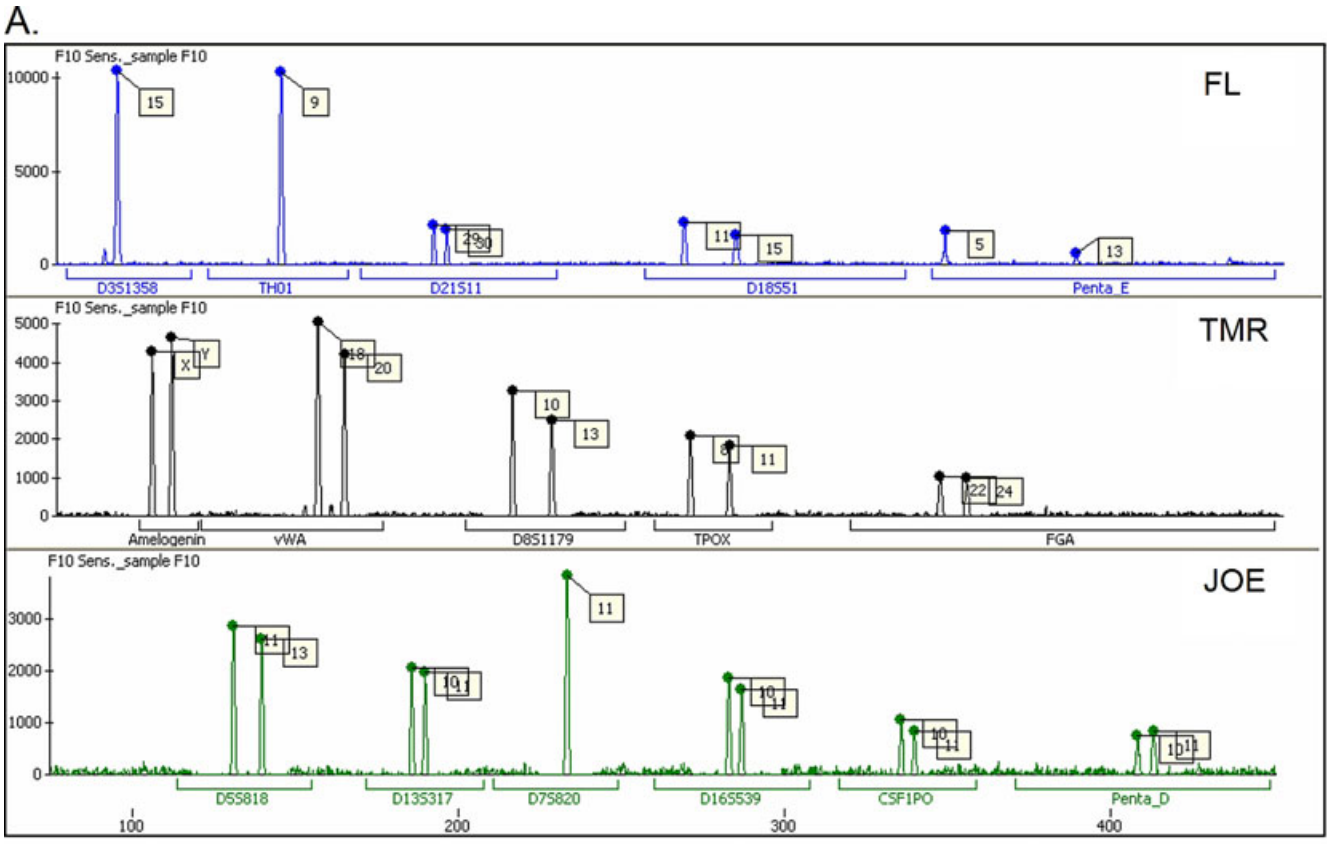

FIG. 3—*PowerPlex® 16 profiles of sensitivity study samples using a single source male DNA amplified with (A) 2 ng of input DNA and (B) 125 pg of input DNA.*

TABLE 4—*Mixture study.*

	Sample #1:Sample #2							
Mixture data	1:0	9:1	3:1	3:2	2:3	1:3	1:9	0:1
Sample 1	14/14	14/14	14/14	14/14	14/14	14/12*	8/14	0/14
Sample 2	0/14	4/14	13/14	14/14	14/14	14/14	14/14	14/14

KEY: 16/16 indicates all of the PowerPlex 16 loci amplified and were correctly typed. All numbers less than 16 indicate the number of loci that were successfully typed.

CSF1PO and Amelogenin loci excluded as alleles were identical for both samples.

*At two of the loci, only one of the minor contributor alleles was observed above peak threshold.

Sensitivity Assays

Evaluation of instrument sensitivity is essential for validation studies and performance checks. Sensitivity assays were performed for both the modified Hjerten-coated chips using a sensitivity series and the pDuramide-coated chips provided by the National Institute of Standards and Technology (NIST) and sensitivity series generated from a DNA sample prepared at VDFS. As demonstrated in Table 3 and displayed in Fig. 3, the sensitivity for the μCAE device was comparable with that reported for commercial capillary electrophoresis instruments (34,35) and to that reported by Yeung et al. (13). Moreover, the coating procedure applied to the microchips did not appear to affect sensitivity.

Mixture Studies

As with sensitivity assays, mixture studies are a critical component to validation work. Table 4 displays the PowerPlex® 16 typing results for a mixture study. The majority of minor contributor alleles were observed at the 3:1 and 1:3 ratios. However, at the 3:1 ratio, a 12 allele at D13S317 was below the threshold for reporting as were a 9 allele at TH01 and an 11 allele at Penta E. These findings are not unexpected and are consistent with previously reported mixture results using commercial detection platforms (33,35). All minor contributor alleles were reported in the Yeung et al. paper for the 1:3 and 3:1 mixture ratios analyzed with the μCAE device. This minor performance difference may be because of the different methods employed for estimating DNA concentration as the mixture samples in the Yeung et al. paper were prepared by NIST using a different methodology for DNA quantitation than that employed at VDFS (13). Collins et al. (36) reported full detection of the minor contributor at the 3:1 and 1:3 ratios; however, the threshold setting was lower than that routinely applied to casework samples (50 rfu) and minor contributor alleles at the stutter position were excluded from analysis, which was not the case in the study reported here. Minor contributor alleles were above the peak threshold at many loci for both the 9:1 and 1:9 ratios as is also consistent with other reports (13,33,35,36). The dynamic coating polymer, pDuramide, was utilized for microchip coating in this experiment, which again indicated that this alternate coating procedure did not adversely impact μCAE device performance.

Nonprobative Sample Analysis

Nineteen nonprobative case samples from five different cases were analyzed using the μCAE device. Nonprobative cases included two sexual assaults, a hit-and-run, aggravated battery, and aggravated robbery/aggravated battery. PowerPlex® 16 profiles produced employing the μCAE device were consistent with profiles produced with the ABI 310 using the same PowerPlex® 16 amplicons (data not shown). Although original DNA typing was conducted on 19 samples using various forensic PCR-based human identification kits, results were in 100% concordance with regards to conclusions that may be drawn from the μCAE device data compared with the conclusions of the original case reported (C. Crouse, personal observations; Fig. 4). As shown in Fig. 4, the minor contributor alleles from the sperm DNA which carried over into the nonsperm fraction are clearly visible and the major profile is identical to the victim profile. This is consistent with the Yeung et al. (13) report which demonstrated that an array of casework could be successfully typed using the μCAE device.

STR Typing Using the PowerPlex® Y System

Mock sexual assault samples consisting of six sperm fractions (1,000 sperm on swab [1K], 10,000 sperm on swab [10K], and 50,000 sperm on swab [50K]) and the six corresponding nonsperm fractions were amplified for PowerPlex® Y and subjected to analysis using both the μCAE device and the ABI 310. Results were concordant between the two instruments and software platforms. However, for a small number of samples with low signal (peak heights close to the 100 rfu threshold on the ABI 310), some peaks could not be labeled if the S/N ratio of 3:1 was applied to data generated using the μCAE device (data not shown). Although no attempts were made to optimize PowerPlex® Y typing of samples using the μCAE device, the majority of the mock sexual assault samples provided STR profiles of similar high quality to those produced by the ABI 310 (Fig. 5).

Yeung et al. (13) clearly demonstrated that the μCAE device performance was consistent with industrial capillary electrophoresis instruments, all without the benefit of commercial, customized software. We report here the successful transfer of this technology from an academic environment to a forensic laboratory, unquestionably demonstrating that this prototype has the capacity to rise to the demands of commercial systems and thus be utilized in other environments. A prototype sample loader, which can pipette 96 samples into the microchip wells within a matter of minutes was not utilized in this current study. Needless to say, the μCAE device has great throughput potential; 96 STR profiles can be separated in less than 30 min. Even when including the time for manual steps in the operation of the μCAE device, the speed and throughput potential should exceed that of the most widely used capillary array instruments. Future studies will incorporate all elements of the existing prototype system, as well as microchips with additional functionalities to provide the most complete picture regarding the time and cost savings potential of the μCAE device.

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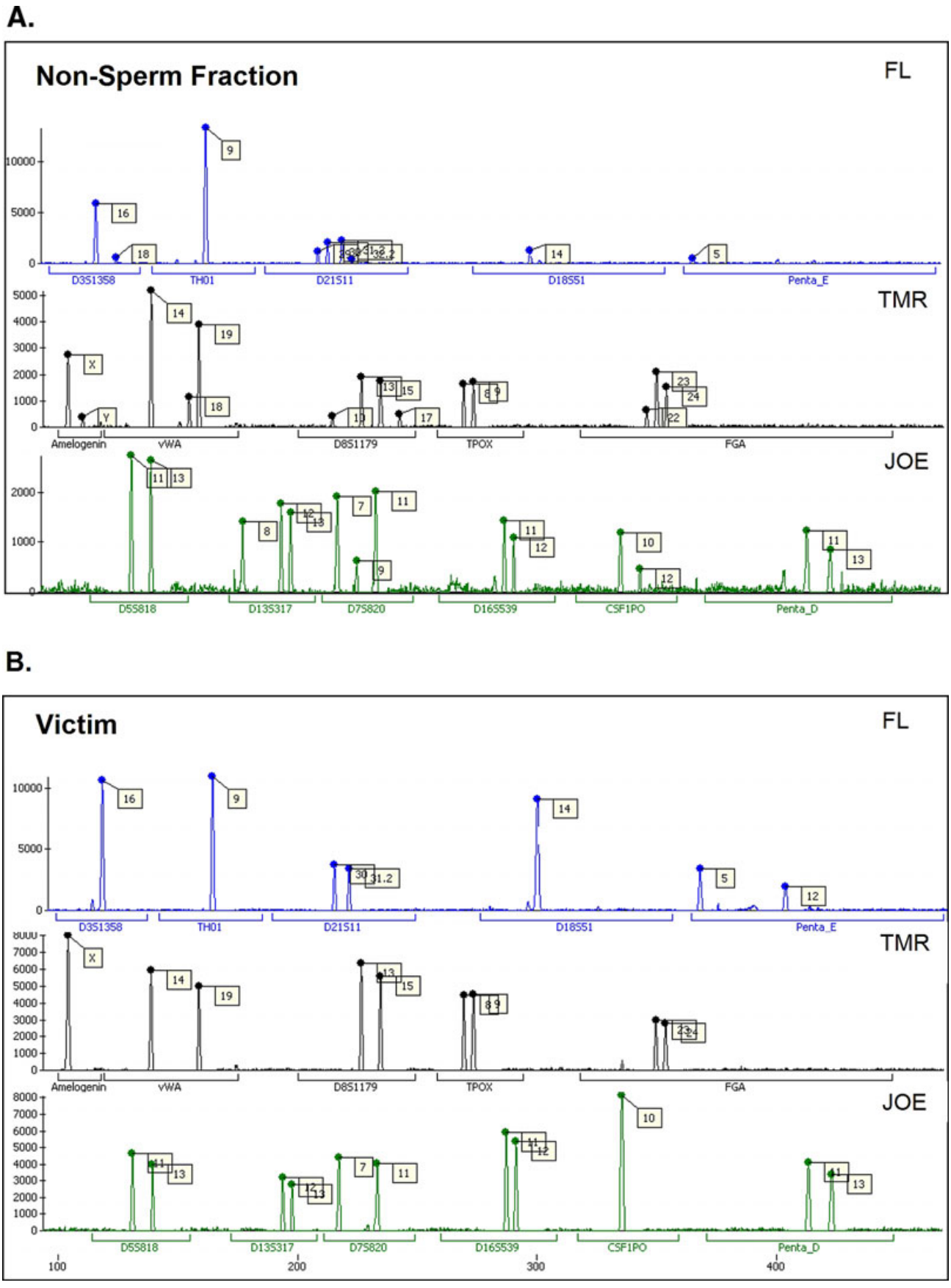

FIG. 4—*Nonprobative sample analysis by the microfabricated capillary array electrophoresis device using PowerPlex® 16. (A) Nonsperm fraction mixed profile. (B) Victim profile.*

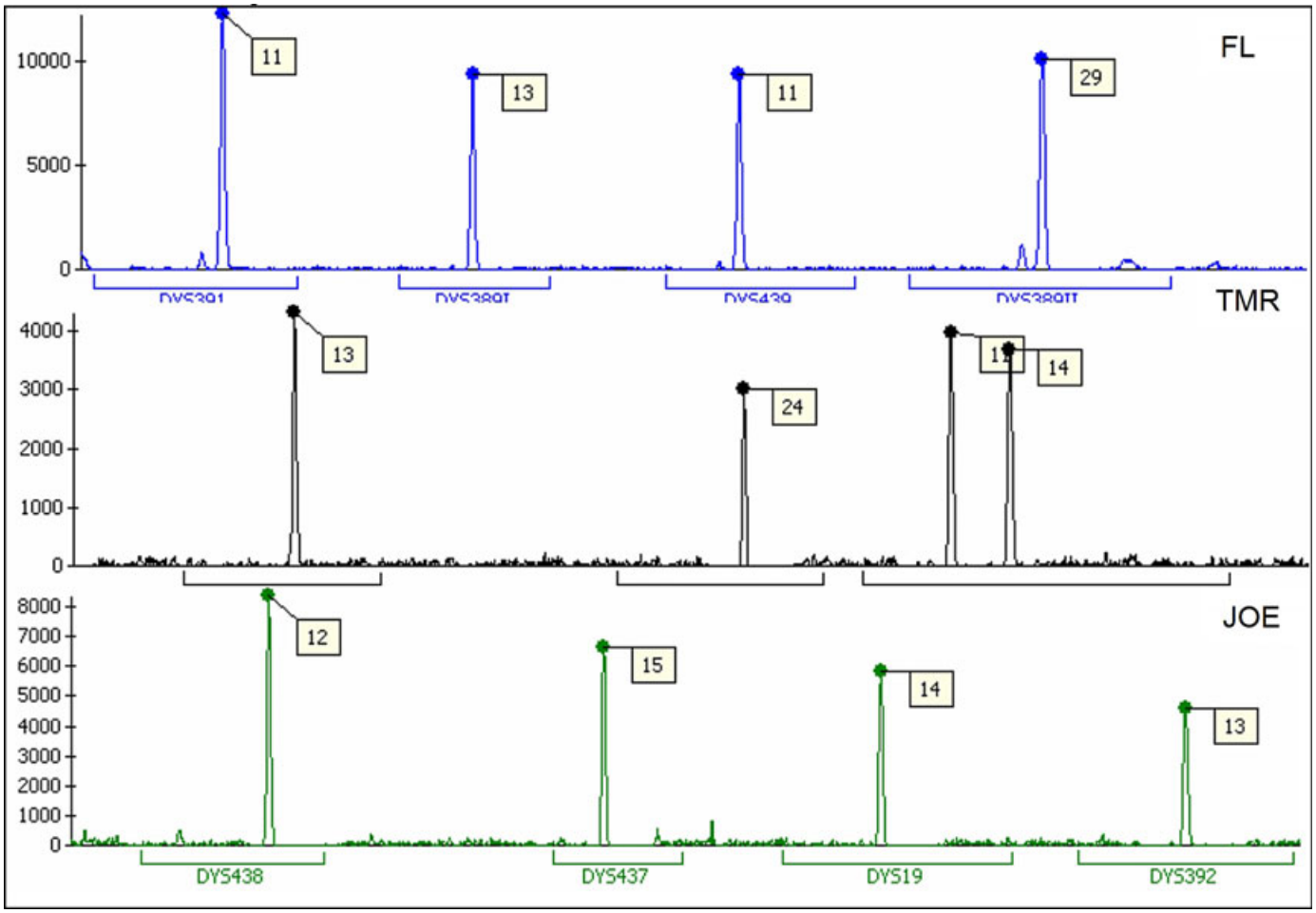

FIG. 5—*PowerPlex® Y profile generated from the sperm fraction of the 50K buccal, mock sexual assault swab.*

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