Results of the 2018 Rapid DNA Maturity Assessment and Earlier Studies

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INTRODUCTION

The forensic DNA typing process involves DNA extraction, PCR amplification, separation and detection, and finally profile interpretation. Integration of these steps into a single instrument is a challenging goal but has become possible in recent years. Several parallel efforts have been made to integrate the forensic STR analysis workflow and utilize a simple "swab in, answer out" process within a single instrument [1-6]. The ANDE (Accelerated Nuclear DNA Equipment) 6C device, developed by ANDE (Waltham, MA) provides users with a fully integrated device to generate full STR profiles using a custom FlexPlex (FP) PCR chemistry containing 27 loci within 90 minutes [1-2]. IntegenX (Pleasanton, CA), which is a subsidiary of Thermo Fisher, developed the RapidHIT 200 and RapidHIT ID devices which utilizes the GlobalFiler Express (GFE) PCR chemistry containing 24 loci and generates full STR profiles in under two hours for the RapidHIT 200 and within 90 minutes for the RapidHIT ID [3-6].

The Applied Genetics Group at the National Institute of Standards and Technology (NIST) has been involved in the testing of integrated rapid DNA instruments since the prototype stage in the fall of 2012 and began robustly testing the maturity of these rapid DNA instruments in 2013. The first rapid DNA maturity assessment, referred to as an interlaboratory study, was performed in August 2013. The study was comprised of three federal laboratories and was conducted to assess the performance of integrated rapid DNA devices within the first year of their release [7].

In 2014, a second rapid DNA maturity assessment was conducted and was comprised of seven independent laboratories. With this study, it was determined the ongoing testing and assessments of the rapid DNA instruments were to be termed "maturity assessments" rather than interlaboratory studies. The 2014 maturity assessment, was conducted to assess the current state of the technology for the 13 CODIS core loci in support of laboratory and future external (non-laboratory-based) rapid DNA instrument implementation [8].

With the expansion of the CODIS core loci from 13 loci to 20 loci in January 2017, a third rapid DNA maturity assessment was proposed to assess the performance of the expanded CODIS core loci [9]. Also, in 2017, the Rapid DNA Act was passed, which amended the DNA Identification Act of 1994, allowing for the implementation of rapid DNA instruments by law enforcement for the DNA testing of arrestees collected in booking station environments. This 2018 rapid DNA maturity assessment was initiated to support the upcoming implementation of rapid DNA technologies outside of the laboratory environment for single-source reference samples [10-11].

The three maturity assessments conducted by NIST focused on the genotyping success for the current CODIS core STR markers (at the time of each assessment), peak height ratio balance, base pair sizing precision, and stutter artifacts for each of the instruments and corresponding STR kit chemistries.

METHODS

Single-source buccal swabs were collected from volunteers under IRB approval from the NIST Humans Subjects Protection Office. Each volunteer was directed to swab the inside of their mouth for ten seconds while rotating the swab. Six to ten buccal swabs were collected sequentially with the donor alternating cheeks between swabs. The swabs were immediately dried in a biosafety cabinet (hood) overnight and were stored at room temperature in the dark prior to shipment to participating laboratories. One buccal swab from each set was extracted and genotyped concordance as a quality control measure. For a buccal swab set to pass quality control, the single extracted buccal swab from the set must have yielded no less than 500 ng of total extracted DNA and produced a full STR profile with conventional laboratory methods.

Three maturity assessments were conducted with blinded single-source reference samples to examine the success of rapid DNA typing technology at each point in time. Sample sets were provided to participants for testing on their rapid instrument(s). Electropherograms (.png), data tracking sheets, .fsa files, and run folders were returned to NIST for review and further analysis. Both automated and manual review of the STR profiles were conducted to assess the success of typing the CODIS core loci. Success for a specific set of STR loci was defined by complete and concordant profiles as called by the expert system software, or via manual review of the electropherogram. The 2013 and 2014 maturity assessments evaluated the success of genotyping the 13 CODIS core loci, while the 2018 maturity assessment evaluated the success of genotyping the 20 CODIS core loci.

In December of 2014, the FBI released an addendum to the Quality Assurance Standards (QAS) allowing the use of rapid DNA instruments in a laboratory setting for uploading single-source reference samples into CODIS [12]. In this addendum, two modalities of analysis were defined; Rapid DNA Analysis and Modified Rapid DNA Analysis. “Rapid DNA Analysis” was defined as the fully automated (hands-free) process of developing a CODIS Core STR profile from a known reference sample without human intervention. “Modified Rapid DNA Analysis” describes the automated (hands-free) process of developing a CODIS Core STR profile from a known reference sample which requires manual interpretation and technical review [12]. These definitions were used to examine the data solely from the 2014 and 2018 maturity assessments, as they were not in existence for the 2013 study.

Year of Study	Rapid DNA Platform	Independent Instruments	Samples Provided Per Lab	STR Typing Chemistry	Samples Attempted Per Lab	Total Samples
2013	ANDE 4C	4	50	PowerPlex 16	200	350
	RapidHIT 200	3			150	
2014	ANDE 4C	5	20	PowerPlex 16	100	280
	RapidHIT 200	6		PowerPlex 16	60	
				GlobalFiler Express	120	
2018	ANDE 6C	5	20	FlexPlex	100	240
	RapidHIT 200	3		GlobalFiler Express	60	
	RapidHIT ID	4		GlobalFiler Express	80	

Table 1: Overview of instruments and samples for all maturity assessments.

2013 Maturity Assessment

Three Federal laboratories participated in this interlaboratory study and received 50 anonymous buccal swabs in five replicates of 10 individuals [7]. Swabs were collected 15 months prior to testing. The ANDE and RapidHIT 200 using the PowerPlex 16 chemistry were operating under the same firmware, software, and script versions, specific to their platform. At this time, the instruments from both vendors were still undergoing developmental upgrades and the automated analysis of electropherograms were not an option, thus samples were analyzed manually for the success of the 13 CODIS core loci. Electropherograms were manually reviewed for genotype concordance to determine success. Stutter and peak height ratios were calculated for the successful samples and reported. Data was analyzed in GeneMapper IDX v1.3 and exported for further analysis in Excel. Stutter percentages and heterozygote balance were evaluated for successfully typed samples.

2014 Maturity Assessment

Seven laboratories, which spanned across U.S. Federal, State, and private laboratories participated in this maturity assessment and received 20 anonymous single-source reference buccal swabs in two replicates of 10 individuals [8]. Swabs were collected 10 months prior to testing. Three RapidHIT 200 instruments ran both the PowerPlex 16 and Globalfiler Express chemistries. Samples were analyzed employing both the Rapid DNA Analysis method and manually with the Modified-Rapid DNA Analysis method for the success of the 13 CODIS core loci [12]. At the time of this study, no Rapid DNA instruments were NDIS approved for automated STR genotype analysis. However, automated allele calls were generated by each instrument and were accepted as an automated mode of Rapid DNA Analysis. Samples which did not produce complete or concordant profiles were analyzed employing Modified Rapid DNA analysis. All data were analyzed in GeneMapper IDX v1.3 and exported for further analysis in

Excel. Stutter percentages, base-pair sizing, and heterozygote balance were evaluated for successfully typed samples.

2018 Maturity Assessment

Nine laboratories, which spanned across U.S. federal and state laboratories, police agencies, and commercial vendors, participated in this interlaboratory study. Each participant received 20 anonymous single-source buccal swabs which were collected five months prior to testing. Replicate buccal swabs were tested across platforms and across participants (n > 5), rather than solely replicated per instrument. Chemistries that support typing of the 20 CODIS core loci were tested on the ANDE 6C (FP), RapidHIT 200 (GFE), and RapidHIT ID (GFE) systems. As the ANDE 6C was the only NDIS approved system at the point of this maturity assessment, it was the only instrument with eligible data for the Rapid DNA analysis method. All samples tested were analyzed with Modified Rapid DNA analysis parameters.

The data from each instrument was manually reviewed to determine correct and concordant allele calls. The ANDE 6C data was imported into GeneMapper IDX v1.5 for analysis with an original analytical threshold of 200 RFU. For profiles that were originally successful in the Rapid DNA analysis of the samples but may have exhibited allele drop out at 200 RFU, the analytical threshold was lowered to 50 RFU for allele calling. The RapidHIT 200 and RapidHIT ID data was reviewed in GeneMarker HID v2.8.2. Both RapidHIT instruments produced a GeneMarker file for analysis which employs dynamic thresholds per locus [4], thus all profiles generated by a RapidHIT instrument were reviewed within GeneMarker rather than re-analyzed within GeneMapper IDX. A heterozygote balance filter of 25% was applied to all samples. After manually interpreting each profile, concordance was checked against the laboratory generated reference profile and success was calculated. Base-pair sizing and peak height ratios were evaluated for successfully typed samples.

RESULTS

Success was measured by complete and concordant genotypes for a specific set of loci (13 CODIS core loci or 20 CODIS core loci) produced by the integrated rapid DNA instruments when compared to genotypes obtained from traditional laboratory methods. As the samples were of high quality and single-source, allelic dropout was not expected (but was observed), hence the requirement of a correct/full genotype as a success metric. Each point in Figure 1 indicates the success of each individual instrument for each of the maturity assessments, while Table 2 is a summary of the average success.

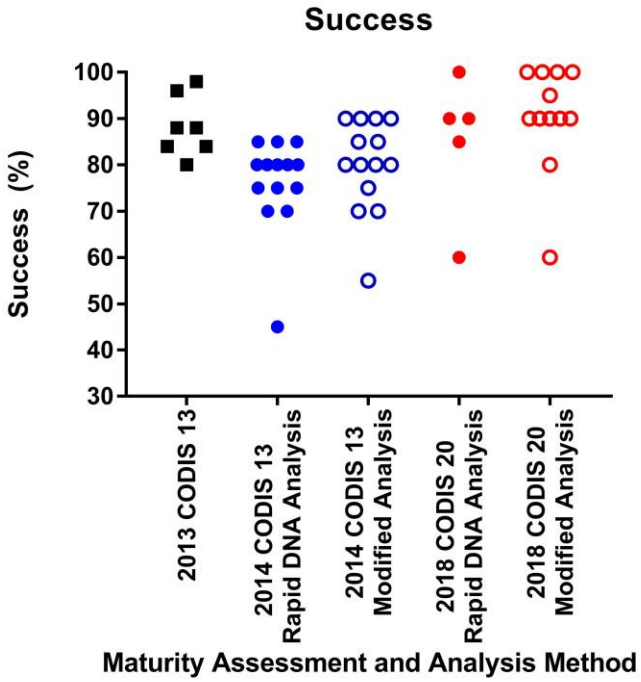

Figure 1: Success across three maturity assessments. Each data point represents the genotyping success for a set of core STR loci on a single rapid DNA instrument.

	Prior to Analysis Definitions	Rapid DNA Analysis		Modified Rapid DNA Analysis	
Year of Study	CODIS 13 Success (%)	CODIS 13 Success (%)	CODIS 20 Success (%)	CODIS 13 Success (%)	CODIS 20 Success (%)
2013	88.3				
2014		76.1	70.0	80.0	75.0
2018			85.0		90.0

Table 2: Average success for the three maturity assessments.

2013 Maturity Assessment Success

Success was reported for the CODIS 13 core STR loci which provided complete and concordant genotypes for the ANDE and RapidHIT 200 instruments (Figure 1). A total of 309 buccal swabs were correctly genotyped the 13 CODIS core loci out of a total of 350 buccal swab samples attempted (Table 2). For the 309 successful samples, heterozygote balance and stutter percentages were calculated on a per instrument basis (Table 3).

2014 Maturity Assessment Success

Success was reported for the 13 CODIS core STR loci and the 20 CODIS core loci (Figure 1 and Table 2). The data point at 45% for Rapid DNA Analysis and 55% for Modified Rapid DNA

Analysis was due to a reagent shipping issue which led to the damage of the reagents and thus lower success than similar instruments.

Table 2 shows the calculated peak height ratios, stutter percentages, and base pair sizing precision for all data. Peak height ratios were calculated for all complete profiles for the combined ANDE and RapidHIT 200 PowerPlex 16 data (n = 118). Median stutter ratio percentages calculated for both the combined PowerPlex 16 dataset and the GlobalFiler Express dataset were within the observed developmental validation range for both PowerPlex 16 and GlobalFiler Express using conventional laboratory techniques [13-14].

2018 Maturity Assessment Success

Success was reported for the 20 CODIS core STR loci for both Rapid DNA Analysis and Modified Rapid DNA Analysis (Figure 1 and Table 2). For Rapid DNA Analysis success, this only includes the ANDE 6C data (n=100), as to date, this is the only rapid DNA system to be NDIS approved for automated rapid DNA analysis. For Modified Rapid DNA analysis, all samples were included in the success (n=240). The data point at 60% for Rapid DNA Analysis was due to the software not employing an onboard allelic ladder after the failure of the ladder on the chip. This led to the entire run "failing", and a loss of five samples. The data point at 60% for the Modified Rapid DNA Analysis was a separate instrument and was due to the loss of one chip from an unknown instrument failure.

Twenty-three samples were unable to be recovered during Modified Rapid DNA analysis of the data generated from this maturity assessment. The modalities of failure included unknown instrument related (n=9), data transfer failures (n=2), partial profiles (n=10), and a single CODIS locus dropout (n=2).

Year of Study	Rapid DNA Platform	STR Typing Chemistry	Average Heterozygote Balance (%)	Median Stutter (%)	Precision (bp)
2013	ANDE	PowerPlex 16	81.6	2.8-13.5	
	RapidHIT 200		92.2	1.5-7.9	
2014	ANDE	PowerPlex 16	87.3	1.5-10.7	0.13
	RapidHIT 200	PowerPlex 16			
		GlobalFiler Express	89.1	1.3-15.6	0.13
2018	ANDE	FlexPlex	83.6		0.15
	RapidHIT 200	GlobalFiler Express	85.3		0.17
	RapidHIT ID	GlobalFiler Express	83.8		

Table 3: Average peak height ratio balance, median stutter ratio, and base pair sizing precision for each maturity assessment.

CONCLUSIONS

Over the past five years, the NIST rapid DNA maturity assessments have provided data to support the success of typing the CODIS core loci with rapid DNA instrumentation. The passing of the Rapid DNA Act of 2017, allows for the processing of single-source reference samples outside of the traditional laboratory setting and in locations such as police booking stations [10-11]. Figure 1 shows the success over the past three rapid DNA maturity assessments for the current rapid DNA systems. This demonstrates the successful and concordant profiles generated for the core 13 and 20 STR loci typed by the fully integrated instruments.

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