Interpol Review of Forensic Biology and Forensic DNA Typing 2016 – 2019
Executive Summary of the following INTERPOL Publication:
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ASCLD Forensic Research Committee
Future Forensics Subcommittee

Forensic Biology and Forensic DNA Typing 2016-2019 (Summarized by Ashley Hall)

1. Introduction. INTERPOL Global DNA Profiling Survey conducted in early 2017 reported that 69 member countries have a national DNA database (https://www.interpol.int/en/How-we-work/Forensics/DNA). The countries responding to the survey reported a combined 35 million DNA profiles contained in the databases. 84 member countries use DNA in police investigations, with 73 performing Y-chromosome STR analysis and 31 using mitochondrial DNA.

2. Core Loci Expansion. In January 2017, the FBI expanded the number of core loci defining the Combined DNA Index System (CODIS) from thirteen to twenty[1] (https://www.fbi.gov/services/laboratory/biometric-analysis/codis). Commercial kits that amplify over 20 STR loci are now used worldwide. The use of a common set of loci increases compatibility of the STR data contained in the various national databases and enables increased international sharing of DNA data. The original core 13 U.S. STR loci are: D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, CSF1PO, FGA, TH01, TPOX, vWA). The seven new U.S. loci are D1S1656, D2S441, D2S1338, D10S1248, D12S391, D19S433, D22S1045. Of these, 15 are commonly included in STR kits employed worldwide: D1S1656, D2S441, D2S1338, D3S1358, D8S1179, D10S1248, D12S391, D16S539, D18S51, D19S433, D21S11, D22S1045, FGA, TH01, and vWA.

3. Rapid Analysis of STR Markers. The Rapid DNA Act of 2017 was signed into U.S. law on August 18, 2017 (https://www.congress.gov/bill/115th-congress/house-bill/510/text). The Act authorizes the FBI Director to "issue standards and procedures for the use of rapid DNA instruments and resulting DNA analyses." Rapid DNA instruments condense the DNA analysis procedure including DNA extraction, amplification, separation, detection, and allele calling to less than 2 hours, the so-called "swab in – profile out." U.S. law enforcement booking station environments have been given the "green-light" to process single-source reference samples, but not crime scene samples. Implementation of the technology to yield faster DNA results is expected to help speed critical decisions in investigations and aid law enforcement. Commercially available rapid DNA instruments include (1) the ANDE 6C (Accelerated Nuclear DNA Equipment 6-color) Rapid DNA System (ANDE, Longmont CO, https://www.ande.com/), (2) the RapidHIT 200 and (3) RapidHIT ID instruments (Thermo Fisher Scientific, South San Francisco CA, https://thermofisher.com/rapidDNA). A number of developmental/internal validation, or evaluation studies have been reported for Rapid instruments. Success rates, calculated as the percentage of CODIS alleles successfully typed (13 in 2016, or 20 in 2017 – 2019), ranged from a low of 50% using GlobalFiler Express on the RapidHT, to a high of 92% on the ANDE 6C with FlexPlex27 (average 79.5% SD 13.2) [2-9]. The FlexPlex27 (ANDE) contains the CODIS core loci and all additional loci required for international databasing [10].

4. Investigative genetic genealogy. Investigative genetic genealogy has drawn international attention with the identification and arrest of alleged "Golden State Killer" Joseph DeAngelo (https://www.nytimes.com/2018/04/26/us/golden-state-killer.html; https://www.nist.gov/blogs/taking-measure/national-dna-day-andbirth-investigative-genetic-genealogy). Subsequently, genealogy databases have aided dozens of other cold case investigations and stimulated discussions surrounding genetic privacy [11,12]. The direct-to-consumer (DTC) genomic industry has grown rapidly in the past few years, with databases amassing ancestry DNA data from millions of individuals seeking to discover their family heritage (https://www.technologyreview.com/s/612880/more-than-26-million-people-have-taken-an-at-homeancestry-test/). Genealogical DNA analysis typically involves interrogation of greater than 500,000 SNP markers, and can reveal connections to distant relatives up to third- or fourth-cousins [13]. Since a majority of the individuals with DTC genetic ancestry results contain a Northern European genetic background, however, these types of searches will not be as effective with individuals from genetic heritages from other parts of the world [14]. Public support of law enforcement use of DTC genetic data has been reported as high as 79% in a survey of 1587 participants, and letter to the editor of the journal *Science* notes several factors that mitigate the threat to privacy, including "genetic genealogy is for lead generation, not conviction"[15].

5. Next-generation sequencing. Next-generation sequencing (NGS), or massively parallel sequencing (MPS), has revolutionized DNA sequencing. The widely used capillary electrophoresis platform measures the overall length of a PCR product, while NGS provides the sequences of STR amplicons and their associated stutter products. Aditionally, both STR and SNP markers can be interrogated in a single assay. The abundance of sequence information has opened the potential for new applications such as biogeographical ancestry, DNA phenotyping, and mixture component resolution. There are two primary MPS platforms currently employed in forensic DNA analysis [16]: (1) MiSeq FGx Forensic Genomics Systems (Illumina, San Diego, CA) and (2) Ion Torrent PGM or Ion S5 (ThermoFisher Scientific, Waltham MA). The top four challenges for implementation of MPS have been identified as (1) lack of consistent nomenclature and reporting standards, (2) lack of compatibility with existing national DNA database infrastructure, (3) lack of population data to support statistical calculations, and (4) lack of an adequate legislative framework [17]. A number of published studies have begun to address these issues [18-21], and the FBI has begun accepting data from approved NGS kits for upload to the U.S. national DNA database (Section 4.4 of the NDIS Operational Procedures Manual at https://www.fbi.gov/file-repository/ndisoperational-procedures-manual.pdf/view).

6. DNA mixture interpretation and probabilistic genotyping software. DNA mixtures are common, and even expected, in many forensic investigations (e.g., sexual assaults, mixed bloodstains, handled items). Deciphering the various components present in a mixture and assigning an appropriate weight to the evidence can be challenging. The past few years have seen an increase in the use of probabilistic genotyping software (PGS) to assist DNA mixture interpretation. The general PGS workflow includes: 1) mixture data generated, 2) level of input data determined by lab via analytical threshold, 3) PGS biological model parameters applied and number of contributors estimated, 4) list of weighted genotype possibilities produced from mixture deconvolution and the propositions set, 5) likelihood ration calculated using the provided allele frequencies and reference profiles. As of July 2019, fifteen PGS software systems were available [22], using either "discrete" ("semi-continuous") models or "continuous" ("fully-continuous") models. Several interlaboratory studies, as well as internal and developmental validations have been completed and published [23-28]. Other published studies have explored the challenges in estimating the number of contributors with low levels of DNA [29], the variation in results with four different continuous

PGS models [30], responses to court admissibility challenges with STRmix [31], and machine learning-based assessment for estimating the number of contributors [32].

7. DNA transfer and activity level evaluations. Activity propositions may be the most appropriate approach in cases with minute quantities of DNA as the focus of the court shifts from questions about the source of the DNA to the mechanism by which it was deposited[33]. Published studies providing greater understanding of the factors affecting DNA transfer fall into two main categories: 1) studying a specific variable, and 2) using simulated casework to evaluate various factors. Shedder status, the effects of multiple donors, secondary transfer, and persistence of DNA proved to be important areas of research. The studies showed that shedder status not only changes in a majority of cases [34], but is also a factor in the relative DNA contribution of two or more people handling the same object[35,36] and in the transfer of self and non-self components to a surface [37]. The simulated casework experiments tended to support these results. In mock assault scenarios, a high number of non-self-alleles supported the implications of shedder status and background DNA on direct and secondary transfer[38]. In the examination of clothing, both wearer and non-wearer contributions could be detected in varying ratios[39], persisting even after laundering in some cases[40]. Other studies employing mock crime scenarios confirmed that trace levels of DNA could be detected in human bite marks[41] and under fingernails after scratching[42]. Finally, RNA profiling showed that approximately 15% of the biological material found originated from non-skin body fluids[43].

A series of comprehensive reviews provide an informative view of the field to date, discussing the mechanism of DNA transfer from a subject [44] and variables affecting transfer of DNA [45], as well as encouraging harmonization and sharing of data[46], and rationalizing how data should be compiled to support its use by practitioners[47].

8. Forensic biology and body fluid identification. Conventional immunochromatographic test strips detect a single body fluid. However, researchers have improved upon this design by constructing a combined test array, based on commercially available tests, to rapidly detect up to five body fluids simultaneously. With this test it was possible to identify the components of a mixture, the test was easily incorporated into standard laboratory work, and its sensitivity and specificity were shown to be comparable to those of conventional strip tests[48].

While the ideal molecular/cellular solution to body fluid identification is not yet available, RNA continues to be the main focus. MicroRNA (miRNA) molecules have high tissue specificity and are resistant to degradation due to their small size[49]. Using differential expression assays are compatible with and complementary to forensic DNA analysis, miRNA analysis has proven applicable to realistic forensic samples, e.g., mixtures, aged and degraded material[50]. Messenger RNA (mRNA) profiling has also proven successful as a body fluid identification technique [51]. HyBeacons, linear oligonucleotides which incorporate fluorescent dyes covalently linked to internal nucleotides, have been evaluated for use in the identification of expressed gene sequences through mRNA profiling. They show a high degree of specificity to the target body fluid mRNA, indicating that there is not a requirement to remove genomic DNA prior to analysis[52]. The 2017 EuroForGen-NOE and EDNAP laboratories completed a collaborative exercise involving massively parallel sequencing of the mRNA transcriptome. They demonstrated moderate-to-high count values in the body fluid or tissue of interest with little-to-no counts in non-target body fluids. Such data could be used in a probabilistic model to predict the origin of stains incorporating quantitative information (NGS read counts) in addition to presence/absence of markers[53,54].

Various other techniques described in the literature include protein markers[55-58], dye-infused Phadebas paper[59], Fourier transform infrared spectroscopy[60], near infrared visualization[61], microbial forensics [62], and methylation analysis [63].

9. DNA phenotyping. Continuing research into the genetic components of age, ancestry, and appearance have improved DNA phenotyping capabilities. Studies have examined the genetics of eye color[64,65], hair shape and darkening with age[66,67], stature[68], eyebrow color[69], and skin color, tanning, and freckling[70]. The HIrisPlex-S system for eye, hair, and skin color prediction has undergone developmental validation studies[71], and is available as an online tool (https://hirisplex.erasmusmc.nl/).

The VISAGE (Visible Attributes Through Genomics) Consortium (http://www.visage-h2020.eu/), an European Union (EU)-funded research and innovation program, examined the regulatory and legal frameworks for phenotyping in the 8 EU member states in their report entitled "The regulatory landscape of forensic DNA phenotyping in Europe" (http://www.visage-h2020.eu/Report_regulatory_landscape_FDP_in_Europe2.pdf). Critical VISAGE researcher reviews highlight epigenetics[72-74], spatial distribution of eye and hair pigmentation[75], and stakeholder identified issues associated with forensic DNA phenotyping[76].

10. Privacy and ethical issues

A report entitled "Establishing Best Practice for Forensic DNA Databases" (September 2017), was prepared by Forensic Genetics Policy Initiative (http://dnapolicyinitiative.org/report/).

The social and ethical responses to the history of innovations in forensic genetics and their application to criminal investigations were reviewed in a 2017 article in *Forensic Science Reviews*[77], and a study of what influences public views on forensic DNA testing in the criminal field was reported in 2017 in *Human Genomics*[78].

A 2016 report in *Forensic Science International: Genetics* discussed the tension between the potential of technology and the ethics of increasing the power of the state, albeit to prevent crime[79].

An article in *Forensic Science International: Genetics* in 2018 looks at the adoption of phenotyping from a privacy perspective, using this to inform and critique the application of a Privacy Impact Assessment to this emerging technology[80].

The journal *Developing World Bioethics* contained an article that examined the numerous ethical and social considerations associated with research investigating the discriminatory power of genetic markers for trait prediction in South Africa[81].

11. Guidance documents. A growing number of standards and guidance documents are being published by various organizations around the world. They are summarized and referenced in Table 3 of the Interpol publication [82], which is included as Appendix 1 of this document.

11.1. SWGDAM activities. Scientific Working Group on DNA Analysis Methods (SWGDAM) is a forum for discussing, sharing, and evaluating forensic biology methods, protocols, training, and research (see https://www.swgdam.org/). SWGDAM meets semiannually in January and July.

11.2. OSAC activities. The Organization of Scientific Area Committees for Forensic Science (OSAC) was launched in 2014. OSAC is administered by the National Institute of Standards and Technology (NIST) to facilitate development of technically-sound documentary standards and adoption of these standards across the forensic science community (see https://www.nist.gov/topics/organization-scientific-area-committees-forensic-science). Publications include:

- Monthly standards bulletins:https://www.nist.gov/topics/forensic-science/organization-scientific-area-committees-osac/osac-newsroom/osac-standards).
- Quarterly newsletter: https://www.nist.gov/topics/forensic-science/organization-scientific-area-committees-osac/osac-newsroom/osac-newsletter)
- OSAC Registry of Approved Standards: https://www.nist.gov/topics/forensic-science/organization-scientific-area-committees-osac/osac-registry/osac-approved
- OSAC Biological Methods Subcommittee work products: https://www.nist.gov/topics/forensic-science/biological-methods-subcommittee
- OSAC Biological Data Interpretation and Reporting Subcommittee work products: https://www.nist.gov/topics/forensic-science/biological-data-interpretation-and-reporting-subcommittee
- OSAC Wildlife Forensics Subcommittee work products: https://www.nist.gov/topics/forensic-science/wildlife-forensics-subcommittee)
- OSAC Lexicon (contains forensic DNA terms defined by the Biology/DNA Scientific Area Committee http://lexicon.forensicosac.org/Term/Home/Index)

11.3. ASB activities. The twelve consensus bodies of the AAFS Standards Board (ASB) published documents describing standards and best practices: https://www.asbstandardsboard.org/published-documents/). The DNA Consensus Body published a document describing mixture interpretation protocols: https://asb.aafs.org/wp-content/uploads/2018/09/020_Std_e1.pdf

11.4. ENFSI DNA working group activities. (http://enfsi.eu/about-enfsi/structure/working-groups/dna/). Recent documents are captured in table 3 of the Interpol summary[82] and in Appendix 1 of this document.

12. Contamination avoidance and DNA success rates. Links to published guidance on contamination avoidance is included in table 3 of the Interpol summary[82] and in Appendix 1 of this document. Additionally, research has highlighted additional concerns increased sensitivity raises for contamination avoidance. Studies considered several possible sources of contamination such as fingerprint brushes [83]or gloves[84], focused on issues in police stations and laboratories[85], and investigated the need for elimination databases to reduce the impact of contamination[86]. A case history of miscarriage of justice[87] and a comprehensive study of transfer of DNA within a Biology laboratory[88] are also included.

Knowledge of DNA success rates can assist in optimizing sample selection criteria and "a thorough selection of DNA traces for analysis, based on DNA success rates, will lead to fewer unnecessary analysis activities and will therefore shorten turnaround times and reduce backlogs"[89].

13. Recent special issues and review articles of note

Electrophoresis: Volume 37, Issue 21 (October 2016) "Forensic Analysis" (https://onlinelibrary.wiley.com/toc/15222683/2016/37/21).

Electrophoresis: Volume 39, Issue 21 (November 2018) "Novel Applications of Massively Parallel Sequencing (MPS) in Forensic Analysis" (https://onlinelibrary.wiley.com/toc/15222683/2018/39/21).

Genes: "Forensic Genomics" containing 11 articles published between November 2017 and December 2018 (https://www.mdpi.com/journal/genes/special_issues/Forensic_Genomics).

Forensic Science International: Genetics: "Trends and Perspectives in Forensic Genetics" containing 11 articles from the September 2018, November 2018, and January 2019 issues of the journal (https://www.journals.elsevier.com/forensic-science-international-genetics/special-issues)

Forensic Science International: "Cold Cases" containing articles from the May, June, July, and August 2019 issues of the journal.

Analytical Chemistry: 2019, Volume 91, Issue 1, pp673–688, "Forensic DNA Analysis"[90]

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APPENDIX 1

Table 3. Guidance documents related to forensic DNA published from 2016 to 2019[82].

SWGDAM December 2016
Recommendations for the Efficient DNA Processing of Sexual Assault Evidence Kits
https://docs.wixstatic.com/ugd/4344b0_4daf2bb5512b4e2582f895c4a133a0ed.pdf

SWGDAM December 2016
Validation Guidelines for DNA Analysis Methods
https://docs.wixstatic.com/ugd/4344b0_813b241e8944497e99b9c45b163b76bd.pdf

SWGDAM January 2017 Contamination Prevention and Detection Guidelines for Forensic DNA Laboratories
https://docs.wixstatic.com/ugd/4344b0_c4d4dbba84f1400a98eaa2e48f2bf291.pdf

SWGDAM January 2017 Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories
https://docs.wixstatic.com/ugd/4344b0_50e2749756a242528e6285a5bb478f4c.pdf

SWGDAM July 2018 Recommendations of the SWGDAM Ad Hoc Working Group on Genotyping Results Reported as Likelihood Ratios
https://docs.wixstatic.com/ugd/4344b0_dd5221694d1448588dcd0937738c9e46.pdf

SWGDAM April 2019 Addendum to "SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories" to Address Next Generation Sequencing
https://docs.wixstatic.com/ugd/4344b0_91f2b89538844575a9f51867def7be85.pdf

SWGDAM April 2019 Interpretation Guidelines for Mitochondrial DNA Analysis by Forensic DNA Testing Laboratories
https://docs.wixstatic.com/ugd/4344b0_f61de6abf3b94c52b28139bff600ae98.pdf

SWGDAM January 2018 Quality Assurance Standards for Forensic DNA Testing Laboratories (draft)
https://docs.wixstatic.com/ugd/4344b0_d4c50d6204b240d3ab23e388b5f6591a.pdf

SWGDAM February 2019
FBI Quality Assurance Standards Audit for Forensic DNA Testing Laboratories (draft)
https://docs.wixstatic.com/ugd/4344b0_7b03780db7244a5b9a93b3bdd59345b5.pdf

SWGDAM February 2019
Quality Assurance Standards for DNA Databasing Laboratories (draft)
https://docs.wixstatic.com/ugd/4344b0_bf68274461f3425888adce9399115099.pdf

SWGDAM February 2019
FBI Quality Assurance Standards Audit for DNA Databasing Laboratories (draft)
https://docs.wixstatic.com/ugd/4344b0_990aee2783af4a82b4d21358e0bd1c53.pdf

US DOJ September 2018
Department of Justice Uniform Language for Testimony and Reports for Forensic Autosomal DNA Examinations Using Probabilistic Genotyping Systems
https://www.justice.gov/olp/page/file/1095961/download

US DOJ September 2018
Department of Justice Uniform Language for Testimony and Reports for Forensic Mitochondrial DNA Examinations
https://www.justice.gov/olp/page/file/1095966/download

US DOJ September 2018
Department of Justice Uniform Language for Testimony and Reports for Forensic Y-STR Data Examinations
https://www.justice.gov/olp/page/file/1095976/download

US DOJ September 2018
Department of Justice Uniform Language for Testimony and Reports for Forensic Serological Examinations
https://www.justice.gov/olp/page/file/1095971/download

ASB September 2018
Standard for Validation Studies of DNA Mixtures, and Development and Verification of a Laboratory's Mixture Interpretation Protocol
https://asb.aafs.org/wp-content/uploads/2018/09/020_Std_e1.pdf

ISO/TC 272 February 2016
ISO 18385:2016 Minimizing the Risk of Human Contamination in Products Used to Collect, Store and Analyze Biological Material for Forensic Purposes e Requirements
https://www.iso.org/standard/62341.html?browse¼tc

ISO/TC 272 August 2018 ISO 21043e1:2018 Forensic Sciences e Part 1: Terms and Definitions
https://www.iso.org/standard/69732.html?browse¼tc

ISO/TC 272 August 2018 ISO 21043e2:2018 Forensic Sciences e Part 2: Recognition, Recording, Collecting, Transport and Storage of Items
https://www.iso.org/standard/72041.html?browse¼tc

ISO/CASCO November 2017
ISO/IEC 17025:2017 General Requirements for the Competence of Testing and Calibration Laboratories
https://www.iso.org/standard/66912.html

ENFSI May 2017 Best Practice Manual for the Internal Validation of Probabilistic Software to Undertake DNA Mixture Interpretation
http://enfsi.eu/wp-content/uploads/2017/09/Best-Practice-Manual-for-the-internal-validation-of-probabilistic-software-to-undertake- DNA-mixture-interpretation-v1.docx.pdf

ENFSI DNA WG April 2017 DNA Contamination Prevention Guidelines
http://enfsi.eu/wp-content/uploads/2017/09/DNA-contamination-prevention-guidelines-v2.pdf

ENFSI DNA WG April 2017 DNA Database Management Review and Recommendations
http://enfsi.eu/wp-content/uploads/2017/09/DNA-databasemanagement-review-and-recommendatations-april-2017.pdf

UKFSR October 2017
Codes of Practice and Conduct for Forensic Science Providers and Practitioners in the Criminal Justice System (Issue 4)
https://www.gov.uk/government/publications/forensic-science-providers-codes-of-practice-and-conduct-2017

UKFSR March 2016 Validation: Use of Casework Material (FSR-P-300)
https://www.gov.uk/government/publications/protocol-using-casework-material-for-validation-purposes

UKFSR July 2016 Sexual Assault Referral Centres and Custodial Facilities: DNA Anti-Contamination
https://www.gov.uk/government/publications/sexual-assault-referral-centres-and-custodial-facilities-dna-anti-contamination

UKFSR July 2016 Crime Scene DNA: Anti-Contamination Guidance
https://www.gov.uk/government/publications/crime-scene-dna-anti-contamination-guidance

UKFSR September 2018
Software Validation for DNA Mixture Interpretation (FSR-G-223)
https://www.gov.uk/government/publications/software-validation-for-dna-mixture-interpretation-fsr-g-223

UKFSR October 2018
DNA Mixture Interpretation (FSR-G-222)
https://www.gov.uk/government/publications/dna-mixture-interpretation-fsr-g-222

ISFG DNA Commission
January 2016 Massively parallel sequencing of forensic STRs: Considerations … on minimal nomenclature requirements
https://www.isfg.org/files/d5ccd549ee232596c75ad8a0b435190e7dba3035.parson2016_str.recommendations.pdf

ISFG DNA Commission June 2016 Recommendations … on quality control of autosomal short tandem repeat allele frequency databasing (STRidER)
https://www.isfg.org/files/db9864824b44997f1014a62a0321f0d25ef6cf98.bodner2016_strider.pdf
ISFG DNA Commission
September 2016
Recommendations on the validation of software programs performing biostatistical calculations for forensic genetic applications

https://www.isfg.org/files/225be64835df624d1ddac70b95a2e7354f916fbb.coble_software_validation_fsigen2016.pdf

ISFG DNA Commission
May 2017 Guidelines on the use of X-STRs in kinship analysis
https://www.isfg.org/files/eea3394d1595b83aeb59e093725518fb94691e78.tillmar2017_x.str.recommendations.pdf

ISFG DNA Commission
July 2018 Assessing the value of forensic biological evidence e guidelines highlighting the importance of propositions. Part I: evaluation of DNA profiling comparisons given (sub-) source propositions

OSAC Ongoing Numerous documents under development