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# Recent advances in forensic biology and forensic DNA typing: INTERPOL review 2019–2022

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ABSTRACT

This review paper covers the forensic-relevant literature in biological sciences from 2019 to 2022 as a part of the 20th INTERPOL International Forensic Science Managers Symposium. Topics reviewed include rapid DNA testing, using law enforcement DNA databases plus investigative genetic genealogy DNA databases along with privacy/ethical issues, forensic biology and body fluid identification, DNA extraction and typing methods, mixture interpretation involving probabilistic genotyping software (PGS), DNA transfer and activity-level evaluations, next-generation sequencing (NGS), DNA phenotyping, lineage markers (Y-chromosome, mitochondrial DNA, X-chromosome), new markers and approaches (microhaplotypes, proteomics, and microbial DNA), kinship analysis and human identification with disaster victim identification (DVI), and non-human DNA testing including wildlife forensics. Available books and review articles are summarized as well as 70 guidance documents to assist in quality control that were published in the past three years by various groups within the United States and around the world.

## 1. Introduction

This review explores developments in forensic biology and forensic DNA analysis of biological evidence during the years 2019–2022. In some cases, there may be overlap with 2019 articles mentioned in the previous INTERPOL review covering 2016 to 2019 [1]. This review includes books and review articles, published guidance documents to assist in quality control, rapid DNA testing, using law enforcement DNA databases plus investigative genetic genealogy DNA databases along with privacy/ethical issues, forensic biology and body fluid identification, DNA extraction and typing methods, mixture interpretation involving probabilistic genotyping software (PGS), DNA transfer and activity level evaluations, next-generation sequencing (NGS), DNA phenotyping, lineage markers (Y-chromosome, mitochondrial DNA, X-chromosome), new markers and approaches (microhaplotypes, proteomics, and microbial DNA), kinship analysis and human identification with disaster victim identification (DVI), and non-human DNA testing including wildlife forensics.

Multiple searches, using the Scopus (Elsevier) and Web of Science (Clarivate) databases, were conducted in the first half of 2022 with "forensic" and "DNA" or "biology" and "2019 to 2022" as search options. Over 4000 articles were returned with these searches. Through visual examination of titles and authors, duplicates were removed, and articles sorted into 32 subcategories to arrive at a list of almost 2000 publications that were supplemented throughout the remainder of the year as this review was being prepared. The tables of contents for non-indexed journals, such as *WIRES Forensic Science*, *Journal of Forensic Identification*, and *Forensic Genomics* were also examined to locate potentially relevant articles.

For example, a Scopus search conducted on June 13, 2022, using "forensic DNA" and "2019 to 2022" found a total of 3059 documents. Table 1 lists the top ten journals from this search. The *Forensic Science International: Genetics Supplement Series* (see row #4 in Table 1) provides the proceedings of the International Society for Forensic Genetics (ISFG) meeting held in Prague in September 2019. This volume contains 914 pages with 347 articles (although only 172 showed up in the Scopus search) that are freely available at https://www.fsigeneticssup.com/[2]. Thus, searches conducted with one or even multiple databases (e.g., Scopus and Web of Science) may not be comprehensive or exhaustive.

### 1.1. Books, special issues, and review articles of note

Books published during the period of this review relating to forensic biology and forensic DNA include *Essential Forensic Biology, Third Edition* [3], *Principles and Practices of DNA Analysis: A Laboratory Manual for Forensic DNA Typing* [4], *Forensic DNA Profiling: A Practical Guide to*

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**Table 1**
Top ten journals with forensic DNA articles published from 2019 to 2022 based on a Scopus search on June 13, 2022.

| Ranking | Journal Titles | Number of Articles on Forensic DNA (2019–2022) |
|---|---|---|
| 1 | *Forensic Science International: Genetics* | 429 |
| 2 | *International Journal of Legal Medicine* | 277 |
| 3 | *Forensic Science International* | 188 |
| 4 | *Forensic Science International: Genetics Supplement Series* | 172 |
| 5 | *Journal of Forensic Sciences* | 109 |
| 6 | *Legal Medicine* | 79 |
| 7 | *Science & Justice* | 65 |
| 8 | *Australian Journal of Forensic Sciences* | 64 |
| 9 | *Genes* | 55 |
| 10 | *Scientific Reports* | 52 |

*Assigning Likelihood Ratios* [5], *Forensic Practitioner's Guide to the Interpretation of Complex DNA Profiles* [6], *Silent Witness: Forensic DNA Evidence in Criminal Investigations and Humanitarian Disasters* [7], *Mass Identifications: Statistical Methods in Forensic Genetics* [8], *Probability and Forensic Evidence: Theory, Philosophy, and Applications* [9], *Interpreting Complex Forensic DNA Evidence* [10], *Understanding DNA Ancestry* [11], *Understanding Forensic DNA* [12], and *Handbook of DNA Profiling* [13]. The 2022 *Handbook of DNA Profiling* spans two volumes and 1206 pages with 54 chapters from 115 contributors representing 17 countries.

Over the past three years, several special issues on topics related to forensic biology were published in *Forensic Science International: Genetics* and *Genes*. These special issues were typically collated virtually rather than physically as invited articles were published online over some period of time and then bundled together virtually as a special issue. Some of these review articles or a set of special issue articles are open access (i.e., the authors paid a publication fee so that the article would be available online for free to readers).

During the time frame of this INTERPOL DNA review, *FSI Genetics* published two special issues: (1) "Trends and Perspectives in Forensic Genetics" (editor: Manfred Kayser)[1] with nine review and two original research articles published between September 2018 and January 2019, and (2) "Forensic Genetics – Unde venisti et quo vadis?" [Latin for "where did you come from and where are you going?"] (editor: Manfred Kayser) with nine articles published in 2021 and early 2022 and likely two more before the end of 2022. Topics for review articles in these special issues include DNA transfer [14], probabilistic genotyping software [15], microhaplotypes in forensic genetics [16], investigative genetic genealogy [17], forensic proteomics [18], distinguishing male monozygotic twins [19], and using the human microbiome for estimating post-mortem intervals and identifying individuals, tissues, or body fluids [20,21]. All of these topics will be discussed later in this article.

A *Genes* special issue "Forensic Genetics and Genomics" (editors: Emiliano Giardina and Michele Ragazzo)[2] published 11 online articles plus an editorial from April 2020 to January 2021 while another *Genes* special issue "Forensic Mitochondrial Genomics" (editors: Mitch Holland and Charla Marshall)[3] compiled 11 articles from February 2020 to April 2021. An "Advances in Forensic Genetics" *Genes* special issue (editor: Niels Morling)[4] included 25 articles shared between April 2021 and May 2022. In July 2022, the *Advances in Forensic Genetics* articles were compiled as a 518-page book.[5] Other *Genes* special issues in development or forthcoming covering aspects of forensic DNA and requesting potential manuscripts by late 2022 or early 2023 include "State-of-the-Art in Forensic Genetics" (editor: Chiara Turchi),[6] "Trends in Population Genetics and Identification—Impact on Anthropology (editors: Antonio Amorim, Veronica Gomes, Luisa Azevedo),[7] "Identification of Human Remains for Forensic and Humanitarian Purposes: From Molecular to Physical Methods" (editors: Elena Pilli, Cristina Cattaneo),[8] "Improved Methods in Forensic and DNA Analysis" (editor: Marie Allen),[9] "Forensic DNA Mixture Interpretation and Probabilistic Genotyping" (editor: Michael Coble)[10], and "Advances in Forensic Molecular Genetics" (editors: Erin Hanson and Claire Glynn).[11] There has been a proliferation of review articles and special issues in this field in the past several years!

A new journal *Forensic Science International: Reports* was launched in November 2019. As of June 2022, it has published 89 articles involving DNA, most of which are descriptions of population genetic data. Likewise, a June 27, 2022, PubMed search with "forensic DNA" and the journal "Genes" found 88 articles – many of which are part of the previously mentioned special issues.

### 1.2. Guidance documents

Numerous documentary standards and guidance documents related to forensic DNA have been published by various organizations around the world. Table 2 lists 70 such documents released in the past three years (2019–2022) in the United States, UK, Australia, and the European Union.

#### 1.2.1. SWGDAM, FBI, and other US DOJ activities

The Federal Bureau of Investigation (FBI) Laboratory funds the Scientific Working Group on DNA Analysis Methods (SWGDAM)[12] to serve as a forum for discussing, sharing, and evaluating forensic biology methods, protocols, training, and research. In addition to creating guidelines on various topics, SWGDAM, which meets semiannually in January and July, provides recommendations to the FBI Director on the Quality Assurance Standards (QAS) used to assess U.S. forensic DNA laboratories involved in the National DNA Index System (NDIS) that perform DNA databasing and forensic casework. New versions of the QAS became effective July 1, 2020.

SWGDAM work products from the timeframe of 2019–2022 (see Table 2) include QAS audit and guidance documents, mitochondrial DNA analysis and short tandem repeat (STR) interpretation guideline revisions related to next-generation sequencing (NGS), training and Y-chromosome interpretation guidelines, a Y-chromosome Haplotype Reference Database (YHRD) update for U.S. laboratories, and reports on investigative genetic genealogy and Y-screening of sexual assault evidence kits. These documents are all accessible online.[13]

[1] https://www.sciencedirect.com/journal/forensic-science-international-genetics/special-issue/10TSDS4360H.
[2] https://www.mdpi.com/journal/genes/special_issues/Forensic_Genetic.
[3] https://www.mdpi.com/journal/genes/special_issues/forensic_mitochondrial_genomics.
[4] https://www.mdpi.com/journal/genes/special_issues/Advances_Forensic_Genetics.
[5] https://www.mdpi.com/books/pdfdownload/book/5798.
[6] https://www.mdpi.com/journal/genes/special_issues/Bioinformatics_Forensic_Genetics.
[7] https://www.mdpi.com/journal/genes/special_issues/genetics_anthropology.
[8] https://www.mdpi.com/journal/genes/special_issues/Identification_of_Human_Remains.
[9] https://www.mdpi.com/journal/genes/special_issues/Forensic_DNA_analysis.
[10] https://www.mdpi.com/journal/genes/special_issues/Forensic_DNA_Mixture.
[11] https://www.mdpi.com/journal/genes/special_issues/28FBA0G4DH.
[12] See https://www.swgdam.org/.
[13] https://www.swgdam.org/publications.

**Table 2**
Guidance documents related to forensic DNA published from 2019 to 2022. The titles are hyperlinked to available documents. Abbreviations: FBI (Federal Bureau of Investigation), CODIS (Combined DNA Index System), SWGDAM (Scientific Working Group on DNA Analysis Methods), NGS (next generation sequencing), US DOJ (United States Department of Justice), ULTR (Uniform Language for Testimony and Reports), AABB (Association for the Advancement of Blood and Biotherapies), ASB (Academy Standards Board), OSAC (Organization of Scientific Area Committees for Forensic Science), UKFSR (United Kingdom Forensic Science Regulator), ENFSI (European Network of Forensic Science Institutes), NIFS (National Institute of Forensic Science), ISFG (International Society for Forensic Genetics).

| Organization | Publication Date | Guidance Document Title |
|---|---|---|
| FBI | July 2020 | Quality Assurance Standards for Forensic DNA Testing Laboratories |
| FBI | July 2020 | Quality Assurance Standards for DNA Databasing Laboratories |
| FBI | July 2020 | Quality Assurance Standards Audit for Forensic DNA Testing Laboratories |
| FBI | July 2020 | Quality Assurance Standards Audit for DNA Databasing Laboratories |
| FBI | July 2020 | Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and DNA Databasing |
| FBI | Jan 2022 | A Guide to All Things Rapid DNA (13 pages; see also Hares et al., 2020 [22]) |
| FBI | Sept 2019 | Non-CODIS Rapid DNA Considerations and Best Practices for Law Enforcement Use (7 pages) |
| FBI | July 2020 | Rapid DNA Testing for non-CODIS uses: Considerations for Court (5 pages) |
| SWGDAM | Apr 2019 | Mitochondrial DNA Analysis Revisions Related to NGS |
| SWGDAM | Apr 2019 | Addendum to Interpretation Guidelines to Address NGS |
| SWGDAM | Feb 2020 | Overview of Investigative Genetic Genealogy |
| SWGDAM | July 2020 | Report on Y-Screening of Sexual Assault Evidence Kits (SAEKs) |
| SWGDAM | July 2020 | Training Guidelines |
| SWGDAM | Jan 2022 | YHRD Updates for U.S. Laboratories |
| SWGDAM | Mar 2022 | Interpretation Guidelines for Y-Chromosome STR Typing by Forensic DNA Laboratories |
| SWGDAM | Mar 2022 | Supplemental Information for the SWGDAM Interpretation Guidelines for Y-Chromosome STR Typing by Forensic DNA Laboratories |
| US DOJ | July 2019 | Bureau of Justice Assistance (BJA) Triage of Forensic Evidence Testing: A Guide for Prosecutors (49 pages) |
| US DOJ | May 2022 | National Institute of Justice (NIJ) National Best Practices for Improving DNA Laboratory Process Efficiency (104 pages) |
| US DOJ | Mar 2019 | Approved ULTR for the Forensic DNA Discipline – Autosomal DNA with Probabilistic Genotyping (5 pages) |
| US DOJ | Mar 2019 | Approved ULTR for the Forensic DNA Discipline – Mitochondrial DNA (4 pages) |
| US DOJ | Mar 2019 | Approved ULTR for the Forensic DNA Discipline – Y-STR DNA (4 pages) |
| US DOJ | Nov 2019 | Interim Policy on Forensic Genetic Genealogical DNA Analysis and Searching (8 pages [23]; see also Callaghan 2019 [24]) |
| US DOJ | Dec 2019 | Needs Assessment of Forensic Laboratories and Medical Examiner/Coroner Offices: Report to Congress (200 pages) |
| US DOJ | Sept 2021 | NIJ Forensic Laboratory Needs Technology Working Group (FLN-TWG) Implementation Strategies: Next Generation Sequencing for DNA Analysis (29 pages) |
| US DOJ | May 2022 | A Landscape Study Examining Technologies and Automation for Differential Extraction and Sperm Separation for Sexual Assault Investigations (50 pages) |
| US DOJ | Sept 2022 | An Introduction to Forensic Genetic Genealogy Technology for Forensic Science Service Providers (7 pages) |
| ASB | Aug 2019 | Standard for Forensic DNA Analysis Training Programs (ANSI/ASB 022) |
| ASB | Sept 2019 | Standard for Forensic DNA Interpretation and Comparison Protocols (ANSI/ASB 040) |
| ASB | June 2020 | Standard for Training in Forensic DNA Isolation and Purification Methods (ANSI/ASB 023) |
| ASB | July 2020 | Standard for Validation of Probabilistic Genotyping Systems (ANSI/ASB 018) |
| ASB | Aug 2020 | Standard for Internal Validation of Forensic DNA Analysis Methods (ANSI/ASB 038) |
| ASB | Aug 2020 | Standards for Training in Forensic Serological Methods (ANSI/ASB 110) |
| ASB | Aug 2020 | Standard for Training in Forensic Short Tandem Repeat Typing Methods using Amplification, DNA Separation, and Allele Detection (ANSI/ASB 115) |
| ASB | Aug 2020 | Standard for Training in Forensic DNA Quantification Methods (ANSI/ASB 116) |
| ASB | Sept 2020 | Standard for the Developmental and Internal Validation of Forensic Serological Methods (ANSI/ASB 077) |
| ASB | May 2021 | Standard for Training in Forensic DNA Amplification Methods for Subsequent Capillary Electrophoresis Sequencing (ANSI/ASB 130) |
| ASB | Aug 2021 | Standard for Training in Forensic DNA Sequencing using Capillary Electrophoresis (ANSI/ASB 131) |
| ASB | Sept 2021 | Standard for Training in Forensic Human Mitochondrial DNA Analysis, Interpretation, Comparison, Statistical Evaluation, and Reporting (ANSI/ASB 140) |
| OSAC | Mar 2020 | Human Factors in Validation and Performance Testing of Forensic Science (35 pages) |
| OSAC | Apr 2021 | Best Practice Recommendations for the Management and Use of Quality Assurance DNA Elimination Databases in Forensic DNA Analysis (OSAC 2020-N-0007) |
| OSAC | June 2021 | Standard for Interpreting, Comparing and Reporting DNA Test Results Associated with Failed Controls and Contamination Events (OSAC 2020-S-0004) |
| OSAC | May 2022 | Human Forensic DNA Analysis (Current Practice) Process Map (42 pages) |
| UKFSR | Mar 2021 | FSR-C-100, Issue 7 – Codes of Practice and Conduct (2021) |
| UKFSR | Sept 2020 | FSR-C-108, Issue 2 – DNA Analysis: Codes of Practice and Conduct |
| UKFSR | May 2020 | FSR-C-116, Issue 1 – Sexual Assault Examination: Requirements for the Assessment, Collection and Recording of Forensic Science Related Evidence |
| UKFSR | Jan 2021 | FSR-C-118, Issue 1 – Development of Evaluative Opinions |
| UKFSR | Sept 2020 | FSR-G-201, Issue 2 – Validation |
| UKFSR | Sept 2020 | FSR-G-202, Issue 2 – The Interpretation of DNA Evidence (Including Low-Template DNA) |
| UKFSR | Sept 2020 | FSR-P-300, Issue 2 – Validation – Use of Casework Material |
| UKFSR | Sept 2020 | FSR-P-302, Issue 2 – DNA Contamination Detection: The Management and Use of Staff Elimination DNA Databases |
| UKFSR | Sept 2020 | FSR-G-206, Issue 2 – The Control and Avoidance of Contamination in Scene Examination involving DNA Evidence Recovery |
| UKFSR | Sept 2020 | FSR-G-207, Issue 2 – The Control and Avoidance of Contamination in Forensic Medical Examinations |
| UKFSR | Sept 2020 | FSR-G-208, Issue 2 – The Control and Avoidance of Contamination in Laboratory Activities involving DNA Evidence Recovery Analysis |
| UKFSR | May 2020 | |

(continued on next page)

**Table 2** (*continued*)

| Organization | Publication Date | Guidance Document Title |
|---|---|---|
| | | FSR-G-212, Issue 1 – Guidance for the Assessment, Collection and Recording of Forensic Science Related Evidence in Sexual Assault Examinations |
| UKFSR | Sept 2020 | FSR-G-213, Issue 2 – Allele Frequency Databases and Reporting Guidance for the DNA (Short Tandem Repeat) Profiling |
| UKFSR | Sept 2020 | FSR-G-217, Issue 2 – Cognitive Bias Effects Relevant to Forensic Science Examinations |
| UKFSR | Sept 2020 | FSR-G-222, Issue 3 – DNA Mixture Interpretation |
| UKFSR | Sept 2020 | FSR-G-223, Issue 2 – Software Validation for DNA Mixture Interpretation |
| UKFSR | Jun 2020 | FSR-G-224, Issue 1 – Proficiency Testing Guidance for DNA Mixture Analysis and Interpretation |
| UKFSR | Mar 2021 | FSR-G-227, Issue 1 – Y-STR Profiling |
| UKFSR | Apr 2021 | FSR-G-228, Issue 1 – DNA Relationship Testing using Autosomal Short Tandem Repeats |
| UKFSR | Apr 2021 | FSR-G-229, Issue 1 – Methods Employing Rapid DNA Devices |
| ENFSI DNA | Apr 2019 | DNA Database Management Review and Recommendations |
| ENFSI DNA | Mar 2022 | Guideline for the Training of Staff in Forensic DNA Laboratories |
| NIFS | Sept 2019 | Case Record Review in Forensic Biology |
| NIFS | Sept 2019 | Empirical Study Design in Forensic Science - A Guideline to Forensic Fundamentals |
| NIFS | Dec 2019 | Transitioning Technology from the Laboratory to the Field - Process and Considerations for the Forensic Sciences |
| AABB | Jan 2022 | Standards for Relationship Testing Laboratories, 15th Edition |
| ISFG DNA Commission | Jan 2020 | Assessing the value of forensic biological evidence – Guidelines highlighting the importance of propositions. Part II: Evaluation of biological traces considering activity level propositions (Gill et al., 2020 [38]) |
| ISFG DNA Commission | June 2020 | Recommendations on the interpretation of Y-STR results in forensic analysis (Roewer et al., 2020 [39]) |

In January 2022, the FBI produced a 13-page guide[14] on rapid DNA testing describing booking station applications and their vision for future integration of crime scene sample analysis and the Combined DNA Index System (CODIS), which builds on a joint position statement published in July 2020 by leaders of U.S. and European groups [22]. In addition, the FBI has shared guidance on their website for non-CODIS use of rapid DNA testing with law enforcement applications[15] and considerations for court.[16]

United States Department of Justice (US DOJ) Uniform Language for Testimony and Reports (ULTRs),[17] contain three ULTRs for the forensic DNA discipline that became effective in March 2019: autosomal DNA with probabilistic genotyping, mitochondrial DNA, and Y-STR DNA. USDOJ also released an interim policy on investigative genetic genealogy in November 2019 [23] along with an opinion piece in the journal *Science* calling for responsible genetic genealogy [24].

Other agencies within US DOJ, namely the Bureau of Justice Assistance (BJA) and the National Institute of Justice (NIJ), published a guide for prosecutors on triaging forensic evidence [25] and best practices for improving DNA laboratory process efficiency [26]. A 200-page report to Congress on the needs assessment of forensic laboratories and medical examiner/coroner offices was released in December 2019 calling for $640 million annually in additional funding to support U.S. forensic efforts [27].

In September 2021, the Forensic Technology Center of Excellence (FTCOE), which is funded by NIJ, published a 29-page implementation strategy on next-generation sequencing for DNA analysis that was written by the NIJ Forensic Laboratory Needs Technology Working Group (FLN-TWG) [28]. In May 2022, FTCOE released a 50-page landscape study examining technologies and automation for differential extraction and sperm separation used in sexual assault investigations [29]. An introduction to forensic genetic genealogy was released in September 2022 [30].

The FTCOE also published a human factors forensic science sourcebook[18] in March 2022 through open access articles in the journal *Forensic Science International: Synergy*. This sourcebook, which has general applicability rather than being specific to forensic DNA analysts, includes an overview article [31] along with articles on personnel selection and assessment [32], the benefits of committing errors during training [33], how characteristics of human reasoning and certain situations can contribute to errors [34], stressors that impact performance [35], and the impact of communication between forensic analysts and detectives using a new metaphor [36].

### 1.2.2. OSAC and ASB activities

The Organization of Scientific Area Committees for Forensic Science (OSAC)[19] is congressionally-funded and administered by the Special Programs Office within the National Institute of Standards and Technology (NIST). OSAC consists of a governing board and over 600 members and associates organized into seven scientific area committees (SACs) and 22 subcommittees. The Biology SAC is divided into human and wildlife forensic biology activities. The Human Forensic Biology Subcommittee[20] focuses on standards and guidelines related to training, method development and validation, data analysis, interpretation, and statistical analysis as well as reporting and testimony for human forensic serological and DNA testing. The Wildlife Forensics Subcommittee[21] works on standards and guidelines related to taxonomic identification, individualization, and geographic origin of non-human biological evidence based on morphological and genetic analyses.

The Academy Standards Board (ASB)[22] is a wholly owned subsidiary of the American Academy of Forensic Sciences (AAFS) and was established as a standards developing organization (SDO). In 2015, ASB was accredited as an SDO by the American National Standards Institute (ANSI). The ASB DNA Consensus Body, with a membership consisting of practitioners, researchers, and lawyers, develops standards and guidelines related to the use of DNA in legal proceedings. Many of the documents developed by ASB were originally proposed OSAC standards or guidelines.

The OSAC Registry[23] is a repository of high-quality and technically-sound standards (both published and proposed) that are intended for implementation in forensic science laboratories. As of July 2022, the OSAC Registry contains 11 standards published by ASB as well as two (2) proposed OSAC standards or best practice recommendations related to

[14] https://www.fbi.gov/file-repository/rapid-dna-guide-january-2022.pdf/view.
[15] https://www.fbi.gov/file-repository/non-codis-rapid-dna-best-practices-092419.pdf/view.
[16] https://www.fbi.gov/file-repository/rapid-dna-testing-for-non-codis-uses-considerations-for-court-073120.pdf/view.
[17] https://www.justice.gov/olp/uniform-language-testimony-and-reports.
[18] https://forensiccoe.org/human_factors_forensic_science_sourcebook/.
[19] https://www.nist.gov/organization-scientific-area-committees-forensic-science.
[20] https://www.nist.gov/organization-scientific-area-committees-forensic-science/human-forensic-biology-subcommittee.
[21] https://www.nist.gov/topics/organization-scientific-area-committees-forensic-science/wildlife-forensics-subcommittee.
[22] https://www.aafs.org/academy-standards-board.
[23] https://www.nist.gov/organization-scientific-area-committees-forensic-science/osac-registry.

human forensic biology. Another four ASB standards and two proposed OSAC standards related to wildlife forensic biology are on the OSAC Registry. The ASB standards issued in the past three years related to human forensic biology cover interpretation and comparison protocols, training in various parts of the process, and validation of forensic serological and DNA analysis methods as well as probabilistic genotyping systems (see Table 2 for names of these documents). A number of other documents[24] related to serological testing methods, assigning propositions for likelihood ratios in forensic DNA interpretations, validation of forensic DNA methods and software, familial DNA searching, management and use of quality assurance DNA elimination databases, setting thresholds, evaluative forensic DNA testimony, and training in use of statistics are in development within OSAC and ASB.

Additional work products of OSAC include (1) a lexicon[25] with 3282 records (although multiple records may exist for the same word, e.g., there are five definitions provided for "validation" from various sources), (2) a 35-page technical guidance document[26] on human factors in validation and performance testing that describes key issues in designing, conducting, and reporting validation research, (3) a listing of research and development needs in forensic science[27] including 18 identified by the OSAC Human Forensic Biology Subcommittee during their deliberations (Table 3), and (4) process maps for several forensic disciplines including a 42-page depiction of current practices and decisions in human forensic DNA analysis released in May 2022 [37]. As a visual representation of critical steps and decision points, a process map is intended to help improve efficiencies and reduce errors, and highlight gaps where further research or standardization would be beneficial. Process maps can assist with training new examiners and enable development of specific laboratory policies or help identify best practices for the field.

**Table 3**
Research and development needs in forensic biology as identified by the OSAC Human Forensic Biology Subcommittee (as of July 2022, see https://www.nist.gov/osac/osac-research-and-development-needs).

| | OSAC Listed R&D Needs |
|---|---|
| 1 | Applications of the Microbiome in DNA Transfer and Human Identification |
| 2 | Assessing DNA Background and Transfer Scenarios in Forensic Casework |
| 3 | Best Practices to Minimize Potential Biases in the Generation and Interpretation of DNA Profiles |
| 4 | Best Practices for Reporting Likelihood Ratios or Other Probabilistic Results in Court |
| 5 | Characterization, Development and Validation of Methods in Single Cell Isolation and Analysis |
| 6 | Characterization, Optimization and Comparison of DNA Sequencing Methods |
| 7 | Characterizing the Presence and Prevalence of Cell-Free DNA |
| 8 | Development of Infrastructure to Compile and Share Raw Electronic Data for Training and Tool Development |
| 9 | Efficiency, Throughput and Speed Improvements in Rapid DNA Instrumentation Through the Development of Direct PCR Methods |
| 10 | Efficient Collection of DNA at the Scene and from Evidence Items |
| 11 | Establishing the Value and Designing a Process for Including Flanking Region SNPs in Massive Parallel Sequencing Based on STRP Casework |
| 12 | Improving the Recovery of Male DNA from Sexual Assault Kits |
| 13 | Methods in Forensic Genealogy |
| 14 | Non-PCR Based Methods for DNA Amplification and/or Detection |
| 15 | Optimization of DNA Extraction for Low Level Samples |
| 16 | Software Solutions for Low Template and High Order DNA Mixture Interpretation in Sequence and Fragment-Based Methods |
| 17 | Software Solutions for Y-STR Mixture Deconvolution |
| 18 | Solutions in Phenotyping and Ancestry Analyses |

#### 1.2.3. UK Forensic Science Regulator

The UK Forensic Science Regulator (UKFSR) oversees forensic science efforts in England, Wales, and Northern Ireland. In March 2021, the Regulator released the seventh issue[28] of the Codes of Practice and Conduct for forensic science providers and practitioners in the criminal justice system. This 114-page document, which has been updated every few years, provides the overall framework for forensic science activities in the UK with other supporting guidance documents on specific areas like DNA analysis or general tasks like validation. In September 2020, a number of the Regulator documents were revised and reissued. As noted in Table 2 (see rows with documents containing "Issue 1" in the title), new guidance documents were also released in the past few years on sexual assault examinations, development of evaluative opinions, proficiency testing for DNA mixture interpretation, Y-STR profiling, DNA relationship testing, and methods employing rapid DNA testing devices. Table 2 lists 20 guidance documents pertinent to forensic biology from the UKFSR.

#### 1.2.4. European Union and Australia

The European Network of Forensic Science Institutes (ENFSI) DNA Working Group published two documents in the past three years: one on DNA database management and the other on training of staff in forensic DNA laboratories (see Table 2). A best practice manual for human forensic biology and DNA profiling is also under development.

The Australian National Institute of Forensic Science (NIFS) published three documents of relevance to forensic biology on case record review, empirical study design, and transitioning technology from the laboratory to the field (see Table 2).

#### 1.2.5. Other international efforts

The Association for the Advancement of Blood and Biotherapies (AABB)[29] published the 15th edition of their Standard for Relationship Testing Laboratories, which became effective on January 1, 2022. This documentary standard was developed by the AABB Relationship Testing Standards Committee and applies to laboratories accredited for paternity testing and other forms of genetic relationship assessment.

The International Society for Forensic Genetics (ISFG) DNA Commission[30] published two articles during the timeframe of this INTERPOL review (see Table 2). In 2020, guidelines and considerations were published on evaluating DNA results under activity level propositions [38]. In addition, the state of the field regarding interpretation of Y-STR results was examined along with different approaches for haplotype frequency estimation using population data – with the Discrete Laplace approach being recommended [39]. Future ISFG DNA Commission efforts will address STR allele sequence nomenclature and phenotyping.

## 2. Advancements in current practices

This section (Section 2) is intended to be law enforcement and practitioner-focused through examination of advances in current practices. The following section (Section 3) is intended to be researcher-focused through emphasis on emerging technologies and new developments. In this section, topics specifically covered include rapid DNA analysis, use of DNA databases to aid investigations (including familial searching, investigative genetic genealogy, genetic privacy and

[24] See https://www.nist.gov/organization-scientific-area-committees-forensic-science/human-forensic-biology-subcommittee.
[25] https://lexicon.forensicosac.org/.
[26] https://www.nist.gov/osac/human-factors-validation-and-performance-testing-forensic-science.
[27] https://www.nist.gov/organization-scientific-area-committees-forensic-science/osac-research-and-development-needs.
[28] https://www.gov.uk/government/publications/forensic-science-providers-codes-of-practice-and-conduct-2021-issue-7.
[29] https://www.aabb.org/standards-accreditation/standards/relationship-testing-laboratories.
[30] https://www.isfg.org/DNA+Commission.

ethical concerns, and sexual assault kit testing), body fluid identification, DNA extraction and typing methods, and DNA interpretation at the sub-source and activity level.

### 2.1. Rapid DNA analysis

Rapid DNA instruments that provide integrated "swab-in-profile-out" results in 90 min or less can be used in police booking station environments and assist investigations outside of a traditional laboratory environment. These instruments were initially designed for analysis of buccal swabs to help speed processing of reference samples associated with criminal cases. Such samples are expected to contain relatively large quantities of DNA from a single contributor. Some attempts to extend the range of sample types to low quantities of DNA or mixtures have been published with various levels of success (see Table 4). Researcher and practitioners from Australia [40–42], Canada [43], China [44], Italy [45], Japan [46,47], and the United States [48–57] have contributed to an increased understanding of rapid DNA testing capabilities and limitations.

The Accelerated Nuclear DNA Equipment (ANDE) 6C (ANDE, Longmont, CO, USA) and the RapidHIT ID (Thermo Fisher Scientific, Waltham, MA, USA) are the current[31] commercially available rapid DNA systems. Each system consists of a swab for introducing the sample, a cartridge or biochip with pre-packed reagents, the instrument, and analysis software with an expert system for automated STR allele calling. Different sample cartridges can be run on each system depending on the sample type and expected quantity of DNA.

For ANDE, the arrestee cartridge (A-Chip), can accommodate up to five samples and is intended for relatively high quantities of DNA typically collected from reference buccal swabs, while the investigative cartridge (I-Chip), can process up to four samples and is intended for lower quantities of DNA that might be present in casework or disaster victim identification samples. Both ANDE cartridges use the FlexPlex27 STR assay that tests 23 autosomal STR loci, three Y-chromosome STRs, and amelogenin to generate data compatible with DNA databases around the world [51]. The RapidHIT ID ACE cartridge and RapidINTEL cartridge serve similar purposes as the ANDE A-Chip and I-Chip using GlobalFiler Express kit markers (21 autosomal STRs, DYS391, a Y-chromosome insertion/deletion marker, and amelogenin) instead of the FlexPlex assay. The ACE sample cartridge uses buccal swabs while the EXT sample cartridge processes DNA extracts [56]. Sensitivity is enhanced in the RapidINTEL cartridge by increasing the number of PCR cycles from 28 to 32 and decreasing the lysis buffer volume from 500 μL to 300 μL compared to the ACE cartridge parameters [46].

With rapid DNA testing's swab-in and answer-out integrated configuration, limited options exist for testing conditions (e.g., either A-Chip or I-Chip with ANDE). Therefore, users should evaluate performance for the sample types they desired to routinely test in their specific environment. Table 4 summarizes recently published studies containing rapid DNA assessments.

National DNA Index System (NDIS) approval has been provided by the FBI Laboratory for accredited forensic DNA laboratories to use either the ANDE 6C or RapidHIT ID Systems (A-Chip and ACE cartridges only)[32] with eligible reference mouth swabs. As noted in Table 2, the FBI.gov website contains three documents related to rapid DNA testing: "Non-CODIS Rapid DNA Considerations and Best Practices for Law Enforcement Use" (7-pages), "Rapid DNA Testing for Non-CODIS Uses: Considerations for Court" (5-pages), and "A Guide to All Things Rapid DNA" (13-pages) in January 2022 to provide information on the topic to

[31] Previously available rapid DNA systems included the RapidHIT 200 from IntegenX and MiDAS (Miniaturized integrated DNA Analysis System) from the Center for Applied NanoBioscience at the University of Arizona.

[32] See https://le.fbi.gov/science-and-lab-resources/biometrics-and-fingerprints/codis/rapid-dna.

**Table 4**
Summary of 20 rapid DNA instrument validation and evaluation studies published from 2019 to 2022. Abbreviations: A-Chip (arrestee cartridge, designed for high-quantity DNA samples), I-Chip (investigative cartridge, designed for low-quantity DNA samples), ACE (arrestee cartridge with GlobalFiler STR markers), RapidINTEL (uses 32 rather than 28 PCR cycles to increase success with low-quantity DNA samples). A-Chip and I-Chip amplify the FlexPlex set of 23 autosomal STRs, three Y-STRs, and amelogenin [51]. ACE and RapidINTEL utilize the GlobalFiler set of 21 autosomal STRs, one Y-STR, one Y-chromosome InDel, and amelogenin.

| Publication | Instrument | Cartridge/Kit | Test Performed and Success Rates Reported |
|---|---|---|---|
| Amick & Swiger 2019 [56] | RapidHIT ID | ACE and EXT | Performed SWGDAM internal validation studies including known and database-type samples, reproducibility, precision, sensitivity, stochastic effects, mixtures, contamination assessment, and concordance studies |
| Carney et al., 2019 [57] | ANDE 6C | A-Chip | Conducted SWGDAM developmental validation (across 6 labs, 2045 swabs, 13 instruments): species specificity, limit of detection, stability, inhibitors, reproducibility, reference material, mixtures, precision, concordance, signal strength, peak height ratio, stutter, non-template addition, resolution, and contamination assessment; **first-pass success rate (1338 samples with 20 CODIS core loci) = 92%**; successfully interpreted >2000 samples with over 99.99% concordant alleles; data package led to receiving NDIS approval in June 2018 |
| Shackleton et al., 2019 [58] | RapidHIT ID | NGM SElect Express | Described development studies that included process optimization, sensitivity, repeatability, contamination checks, inhibition, swab age, concordance, and overall performance; **success rate (124 samples) = 84.5%** gave a full profile |
| Shackleton et al., 2019 [59] | RapidHIT 200 | NGM SElect Express | Performed some protocol adjustments that enhanced slightly the sensitivity with mock crime scene samples (dilutions of blood and cell line DNA) |
| Yang et al., 2019 [53] | MiDAS | PowerPlex ESI 16 Plus | Described protocols for analysis of reference samples with a fully automated integrated microfluidic system (MiDAS), which is not commercially available |
| Romsos et al., 2020 [48] | ANDE 6C, RapidHIT ID, RapidHIT 200 | A-Chip, ACE | Reported results from the July 2018 rapid DNA maturity assessment with multiple instruments organized by NIST; the average success rate for obtaining the 20 CODIS core loci was **85% (n=240)** |
| | ANDE 6C | A-Chip | |

(continued on next page)

**Table 4** (*continued*)

| Publication | Instrument | Cartridge/Kit | Test Performed and Success Rates Reported |
|---|---|---|---|
| Manzella & Moreno 2020 [49] | | | Reported success rates on 54 samples of **88% using ANDE swabs**, **but only 33% with cotton tipped swabs**, like those that may be received during casework processing; with manual interpretation, the CODIS 20 success rate increased to 63% |
| Murakami et al., 2020 [46] | RapidHIT ID | ACE and RapidINTEL | Examined blood and nail clippings from postmortem bodies with varying degrees of decomposition and reported "the device is useful for samples of sufficient quantity and purity, considering post-mortem intervals of up to approximately one week" |
| Ragazzo et al., 2020 [45] | ANDE 6C | A-Chip | Compared results for 104 buccal swabs with rapid and conventional protocols, observed a **97% success rate** and **99.96% concordance with 2800 markers**, and concluded "the ANDE 6C System is robust, reliable, and is suitable for use in human identification for forensic purposes from a single source of DNA" |
| Kitayama et al., 2020 [47] | ANDE 6C | A-Chip and I-Chip | Examined 19 mock DVI samples; "success rates of putrefied DVI samples varied widely between 0% and 20% and 50%–80% depending on cartridge and sample types" and "DVI samples that yielded more than 1 ng/μL of DNA when extracted with conventional protocols were suitable" (success defined as at least 20 CODIS STRs for A-Chip and any 12 out of 20 CODIS STRs for I-Chip) |
| Turingan et al., 2020 [51] | ANDE 6C | I-Chip | Processed 1705 mock crime scene and DVI samples across 17 sample types; with 1299 samples in the accuracy study, **first pass success rate was 91%** (defined by the authors as at least 16 CODIS STRs on the first run) |
| Turingan et al., 2020 [52] | ANDE 6C | A-Chip and I-Chip | Examined tissues and bones from 10 sets of human remains exposed above ground for up to one year; analysis of bone and teeth works best with extended exposure |
| Chen et al., 2021 [44] | RapidHIT ID | RapidINTEL | Performed substrate, sensitivity, precision, contamination, mock inhibition, mixture, concordance, species, and versatility studies; 100% concordance with conventional CE-based DNA analysis across 19 STRs; **91% success rate** |
| | ANDE 6C | | |

**Table 4** (*continued*)

| Publication | Instrument | Cartridge/Kit | Test Performed and Success Rates Reported |
|---|---|---|---|
| Hinton et al., 2021 [43] | | A-Chip and I-Chip | Examined in a technical exploitation workflow; **96% success with buccal swabs (21 of 22); 67% with controlled samples and 16% for uncontrolled samples** |
| Manzella et al., 2021 [50] | ANDE 6C | I-Chip | Examined 7 muscle tissue, 4 pulverized bone exemplars, 9 rib, and 26 teeth samples and concluded "the robustness and consistency of the method still have room for improvement" |
| Martin et al., 2022 [40] | RapidHIT ID | RapidINTEL | Examined 8 touched samples (10 replicates each) containing low quantities of DNA; found that the method "was not suitable for the 12G cartridge, insulated wire, or twine sampling in its current form" |
| Ridgley & Olson 2022 [55] | ANDE 6C | I-Chip | Evaluated a protocol for sexual assault samples; **98 of 144 samples (68%)** "met the instrument metrics for success and resulted in at least a partial profile" (>8 loci) and "could be immediately used without further review" |
| Cihlar et al., 2022 [54] | RapidHIT ID | ACE | Performed validation experiments with concordance, contamination, sensitivity, repeatability, reproducibility, swab reprocessing, stability, inhibition, and mixture studies (253 samples total); **first-pass success rate of 92%** |
| Ward et al., 2022 [41] | RapidHIT ID | RapidINTEL | Assessed performance for mixture interpretation using STRmix v2.8 (can yield orders of magnitude different LR values compared to standard laboratory workflow) |
| Watherston et al., 2022 [42] | ANDE 6C and RapidHIT ID | I-Chip and RapidINTEL | Used donated cadavers with a simulated building collapse scenario; allele recovery varied by sample type and instrument; concluded "further optimization is recommended for highly decomposed and skeletonized human remains" |

law enforcement agencies.

The ENFSI DNA Working Group, SWGDAM, and an FBI Rapid DNA Crime Scene Technology Advancement Task Group co-published a position statement on the use of rapid DNA testing from crime scene samples [22]. These groups emphasized the need to have future rapid DNA systems with (1) methods to identify low quantity, degradation, and inhibition as well as meeting the human quantification requirements shared by SWGDAM and others, (2) the ability to export analyzable raw data for analysis or reanalysis by trained and qualified forensic DNA analysts, (3) an on-board fully automated expert system to accurately flag single-source or mixture DNA profiles requiring analyst

evaluation, (4) improved peak height ratio balance (per locus and across loci) for low-quality and mixture samples "through enhancements in extraction efficiencies, changes in cycling parameters, and/or changes in STR kit chemistries," and (5) published developmental validation studies on a wide variety of forensic evidence type samples with "data-supported recommendations regarding types of forensic evidence that are suitable and unsuitable for use with Rapid DNA technology" [22].

With a likely increase in the capabilities and the availability of rapid DNA systems, investigators will need to decide whether to use this capability onsite in specific situations or to send collected samples to a conventional forensic laboratory for processing at a later time. A group in the Netherlands collaborated with the New York City Police Department Crime Scene Unit and Evidence Collection Team to explore a decision support system [60]. In this study, participants were informed that rapid DNA testing was less sensitive compared to laboratory analysis and that the sample would be consumed, but that results from rapid DNA testing could identify a suspect within 2 h as opposed to waiting an average of 45 days for the laboratory results [presumably due to sample backlogs]. They were also told that a DNA profile obtained with rapid DNA would be acceptable in court. In the end, ">90% of the participants (85 out of 91) saw added value for using a Rapid DNA device in their investigative process …" with "a systematic approach, which consists of weighing all possible outcomes before deciding to use a Rapid DNA analysis device" [60]. The authors note that for such an approach to be successful "knowledge on DNA success rates [with various evidence types] is necessary in making evidence-based decisions for Rapid DNA analysis" [60].

A group in Australia performed a cost-benefit analysis of a decentralized rapid DNA workflow that might exist in the future with instruments placed at police stations around their country [61]. A virtual assessment considered all reference DNA samples collected during a two-month time period at 10 participating police stations in five regions of Australia. Processing times at the corresponding DNA analysis laboratories were calculated based on when the sample was received compared to the day when a DNA profile was obtained for that sample. From the survey conducted, it was estimated that up to 80,000 reference DNA samples are currently processed each year in forensic DNA laboratories across Australia [61].

Consumable costs for conventional DNA testing reagents in Australia were found to range from $17 to $35 whereas the rapid DNA consumable costs were estimated to be $100 per sample along with an anticipated $100,000 instrument cost per police station. Of course, the rate of use is expected to vary based on the number of reference samples collected in that jurisdiction. Since rapid DNA instruments utilize consumable cartridges with expiration dates, it was estimated that a police station would need to process six DNA samples per week to avoid having to discard an expired cartridge and thus increase the overall cost of their rapid DNA testing efforts. The authors of this study conclude "that routine laboratory DNA analysis meets the current needs for the majority of cases … It is anticipated that while the cost discrepancy between laboratory and rapid DNA processing remains high, the uptake of the technology in Australia will be limited [at least for a police booking station scenario]" [61].

Rapid DNA technology can be used in a variety of contexts including some that extend beyond traditional law enforcement. Seven distinct use contexts for rapid DNA capabilities have been described [62]: (1) *evidence processing* at or near crime scenes to generate leads for confirmation by a forensic laboratory, (2) *booking or detection stations* to compare an individual's DNA profile to a forensic database while the individual is still in custody, (3) *disaster victim identification* to permit rapid DNA processing of a victim's family members during their visit to family assistance centers when filing missing persons reports, (4) *missing persons investigations* to quickly process unidentified human remains and/or family reference samples to generate leads for confirmation by a forensic laboratory, (5) *border security* to develop DNA data from detainees for comparison to indices of prior border crossers while the individual is still in custody, (6) *human trafficking and immigration fraud detection* to permit immigration officials to verify family relationship claims, and (7) *migrant family reunification* to allow immigration officials to verify parentage claims and reunite family members separated at the border. Social and ethical considerations have been proposed for each of these use contexts in terms of data collection, data access and storage, and oversight and data protection [62].

One study [47] evaluating buccal swabs and mock disaster victim identification samples drew an important conclusion worth repeating here: "The Rapid DNA system provides robust and automated analysis of forensic samples without human review. Sample analysis failure can happen by chance in both the Rapid DNA system and conventional laboratory STR testing. While re-injection of PCR product is easily possible in the conventional method, this is not an option with the Rapid DNA system. Accordingly, the Rapid DNA system is a suitable choice but should be limited to samples that can easily be collected again if necessary or to samples that are of sufficient amount for repeated analysis. Application of this system to valuable samples such as those related to casework need to be considered carefully before analysis."

### 2.2. *Using DNA databases to aid investigations (national databases, familial searching, investigative genetic genealogy, genetic privacy & ethical concerns, sexual assault kit testing)*

Forensic DNA databases can aid investigations by demonstrating connections between crime scenes, linking a previously enrolled DNA profile from an arrestee or convicted offender to biological material recovered from a crime scene, or aiding identification of missing persons through association of remains with biological relatives. Establishment of these databases requires significant investments over time to enroll data from crime scenes and potential serial offenders or unidentified human remains and relatives of missing persons. This section explores issues around national DNA databases, familial searching, investigative genetic genealogy, and genetic privacy and ethical concerns.

A systematic review regarding the effectiveness of forensic DNA databases looked at 19 articles published between 1985 and 2018 and found most studies support the assumption that DNA databases are an effective tool for the police, society, and forensic scientists [63]. Recommendations have been proposed to make cross-border exchange of DNA data more transparent and accountable with the Prüm system that enables information sharing across the European Union [64]. An analysis of news articles discussing the use of DNA testing in family reunification with migrants separated at the U.S.-Mexico border has been performed [65], and a standalone humanitarian DNA identification database has been proposed [66]. Aspects of international DNA kinship matching were explored to aid missing persons investigations and disaster victim identification processes [67]. A business case was presented for expanded DNA indirect matching using additional genetic markers, such as Y-chromosome STRs, mitochondrial DNA, and X-chromosome STRs, to reveal previously undetected familial relationships [68].

Approaches to transnational exchange of DNA data include (1) creation of an international DNA database, (2) linked or networked national DNA databases, (3) request-based exchange of data, and (4) a combination of these [69]. For example, the INTERPOL DNA database[33] contains more than 247,000 profiles contributed by 84 member countries. The I-Familia global database assists with missing persons identification based on international DNA kinship matching.[34]

#### 2.2.1. *National DNA databases*

Since the United Kingdom launched the first national DNA database

[33] See https://www.interpol.int/How-we-work/Forensics/DNA.
[34] See https://www.interpol.int/How-we-work/Forensics/I-Familia.

in 1995, national DNA databases continue to be added in many countries including Brazil [70,71], India [72], Pakistan [73,74], Portugal [75], and Serbia [76]. A survey of 15 Latin American countries found that 13 of them had some kind of DNA database [77]. The opinions of 210 prisoners and prison officials in three Spanish penitentiary centers were also collected regarding DNA databases [78].

The effectiveness of databases has been debated over the years. Seven key indicators were used in a 2019 examination of the effectiveness of the UK national DNA database. These indicators included (1) *implementation cost* – the financial input required to implement the database system, (2) *crime-solving capability* – the ability of the database to assist criminal justice officials in case resolution, (3) *incapacitation effect* – the ability of the database to reduce crime through the incapacitation of offenders, (4) *deterrence effect* – the preventative potential of the database through deterrence of individuals from committing crime, (5) *privacy protection* – protection of the privacy or civil liberty rights of individuals, (6) *legitimacy* – compliance of the databasing system to the principle of proportionality, and (7) *implementation efficiency* – the time and non-monetary resource required to implement the database system [79].

A follow-up article concluded: "Available evidence shows that while DNA analysis has contributed to successful investigations in many individual cases, its aggregate value to the resolution of all crime is low" [80]. The systematic review of 19 articles on DNA databases cited previously noted "the expansion of DNA databases would only have positive effects on detection and clearance if the offender were already included in the database" [63]. When previous offenders are not already in a law enforcement DNA database to provide a hit to a crime scene profile, efforts are increasingly turning to familial searching and investigative genetic genealogy as described in the following sections.

### 2.2.2. *Familial DNA searching*

Familial DNA searching (FDS) extends the traditional direct matching of STR profiles within law enforcement databases to search for potential close family relationships, such as a parent or sibling, of a profile in the database.[35] FDS typically uses Y-STR lineage testing to narrow the set of candidate possibilities along with other case information such as geographic details of the crime and age of the person(s) of interest. For example, FDS helped solve murder cases in Romania [81] and China [82] by locating the perpetrator through a relative in the DNA database. A survey of 103 crime laboratories in the United States found that 11 states use FDS while laboratories in 24 states use a similar but distinct practice of partial matching [83].

The expansion of the number of STRs from 15 to 20 or 21 helps distinguish between true and false matches during a DNA database search by reducing the number of FDS adventitious matches [84]. Another study noted that the choice of allele frequencies affects the rate at which non-relatives are erroneously classified as relatives and found that using ancestry inference on the query profile can reduce false positive rates [85]. New Y-STR kits have been developed to assist with familial searching [86,87]. FDS of law enforcement databases differs from investigative genetic genealogy in two important ways – the genetic markers and the databases used for searching [88,89].

### 2.2.3. *Investigative genetic genealogy*

In recent years when national DNA databases fail to generate a lead to a potential person of interest, law enforcement agencies have started to utilize the capabilities of investigative genetic genealogy (IGG), also called forensic genetic genealogy (FGG) or forensic investigative genetic genealogy (FIGG), as an approach to locate potential persons of interest in criminal or missing persons cases. For example, a pilot case study in Sweden used IGG to locate the perpetrator of a double murder from 2004 who had evaded detection despite 15 years of various investigation efforts including more than 9000 interrogations and mass DNA screenings of more than 6000 men [90]. Hardly a week goes by without mention in the global media of another cold case being solved with IGG. Since the arrest of Joseph DeAngelo in April 2018 identified as the infamous Golden State Killer using IGG, hundreds of cold criminal and unidentified human remains cases have been resolved [91].

IGG involves examination of about 600,000 single nucleotide polymorphisms (SNPs), rather than the 20 or so STRs used in conventional forensic DNA testing, to enable associations of relatives as distant as third or fourth cousins [17]. IGG relies on a combination of publicly accessible records and the consent of individuals who have uploaded their genetic genealogy DNA profiles to genetic genealogy databases [92]. Multiple reviews and research articles have been published describing current IGG methods, knowledge, and practice along with the effectiveness and operational limits of the technique [17,30,93–97]. IGG works best with high-quality, single-source DNA samples. A case study involving whole genome sequencing of human remains from a 2003 murder victim found that it was possible to perform IGG for identification of the victim in this situation [98].

The four main direct-to-consumer (DTC) genetic genealogy companies, 23andMe (Mountain View, CA), Ancestry (Salt Lake City, UT), FamilyTree DNA (Houston, TX), and My Heritage (Lehi, UT), have DNA data from over 41 million individuals[36] as of July 2022 [97]. Individuals can upload their DTC data to GEDmatch, which is a DNA comparison and analysis website launched in 2010 and purchased in 2019 by Verogen (San Diego, CA). Law enforcement IGG searches are currently permitted with DTC data for individuals who opt into the GEDmatch database or do not opt out of the FamilyTree DNA database [99,100]. Currently most DTC genetic genealogy data comes from the United States and individuals of European origin. A UK study found that 4 of 10 volunteer donors could be identified with IGG including someone of Indian heritage demonstrating that under the right circumstances individuals of non-European origin can be identified [101].

As noted previously in Section 1.2.1, the U.S. Department of Justice released an interim policy guide to forensic genetic genealogical DNA analysis and searching [23], and the FBI Laboratory's chief biometric scientist published an editorial in *Science* calling for responsible genetic genealogy [24]. SWGDAM has provided an overview of IGG that emphasizes the approach being used only after a regular STR profile search of a law enforcement DNA database fails to produce any investigative leads [102]. Policy and practical implications of IGG have been explored in Australia [103] and within the UK as part of probing the perceptions of 45 professional and public stakeholders [104,105].

Four misconceptions about IGG were examined by several members of the SWGDAM group: (1) when law enforcement conducts IGG in a genetic genealogy database, they are given special access to participants' SNP profiles, (2) law enforcement will arrest a genetic genealogy database participant's relatives based on the genetic information the participant provided to the database, (3) IGG necessarily involves collecting and testing DNA samples from a larger number of innocent persons than would be the case if IGG were not used in the investigation, and (4) IGG is or soon will be ubiquitous because there are no barriers to IGG that limit the cases in which it can be conducted [106].

In May 2021, the state of Maryland passed the first law in the United States and in the world that regulates law enforcement's use of DTC genetic data to investigate crimes. A policy forum article in *Science* explained how this new law provides a model for others in this area [107]. Six important features were described: (1) requiring judicial authorization for the initiation of an IGG search, (2) affirming individual control over the investigative use of one's genetic data, (3) establishing strong protections for third parties who are not suspects in the case, (4) ensuring that IGG is available to prove either guilt or innocence, (5)

[35] See https://le.fbi.gov/science-and-lab-resources/biometrics-and-fingerprints/codis#Familial-Searching.

[36] See https://isogg.org/wiki/Autosomal_DNA_testing_comparison_chart.

imposing consequences and fines for violations, and (6) requiring annual public reporting and review to enable informed oversight of IGG methods. However, as of September 2022, these regulations have not been implemented apparently due to lack of resources with these unfunded requirements.[37]

Efforts have been made to raise awareness among defense attorneys about how IGG searches can potentially invade people's privacy in unique ways [108]. Important perspectives on ethical, legal, and social issues have been offered along with directions for future research [109]. These concerns about data privacy, public trust, proficiency and agency trust, and accountability have led to a call for standards and certification of IGG to address issues raised by privacy scholars, law enforcement agencies, and traditional genealogists [110,111] and for an ethical and privacy assessment framework covering transparency, access criteria, quality assurance, and proportionality [112].

#### 2.2.4. Genetic privacy and ethical concerns

Two important topics are considered in this section: (1) do the genetic markers used in traditional forensic DNA typing reveal more than identity and therefore potentially impact privacy of the individuals tested? and (2) are samples collected and tested according to ethical principles?

Forensic DNA databases utilize STR markers that were intentionally selected to avoid phenotypic associations. An extensive review of the literature examined 107 articles associating a forensic STR with some genetic trait and found "no demonstration of forensic STR variants directly causing or predicting disease" [113]. A study of the potential association of 15 STRs and 3 facial characteristics on 721 unrelated Han Chinese individuals also found "scarcely any association between [the] STRs with studied facial characteristics" [114].

In 2021, the American Type Culture Collection (ATCC) published a standard for authentication of human cell lines using DNA profiling with the 13 CODIS STR markers [115]. This use of forensic STR markers for biospecimen authentication led a bioethicist and a law professor to write a policy forum article in *Science* titled "Get law enforcement out of biospecimen authentication" [116]. The authors of this policy forum believe that using the same genetic markers could potentially: (1) undermine efforts to recruit research participants from historically marginalized and excluded groups that are underrepresented in research, (2) risk drawing law enforcement interest in gaining access to these research data, and (3) impose additional potential harms on already vulnerable populations, particularly children. Instead they advocate for using non-CODIS STRs or a new SNP assay to distinguish biospecimens in repositories, something done recently at the Coriell Institute for Medical Research with six new STR markers [117]. A responsive letter to the editor regarding this policy forum article expressed that "their proposal could potentially create artificial silos between genomic data in the justice system and in biomedical research, making it inefficient and ultimately counterproductive" [118]. The authors of the original article responded that "the risk of attracting law enforcement interest to research data increases when the data are available in a recognizable way" [119].

Modern scientific research seeks to protect the dignity, rights, and welfare of research participants by following ethical requirements. Six forensic science journals over the time period of 2010–2019 were examined for their reporting of ethical approval and informed consent in original research using human or animal subjects [120]. These journals were *Forensic Science International: Genetics*, *Science & Justice*, *Journal of Forensic and Legal Medicine*, the *Australian Journal of Forensic Sciences*, *Forensic Science International*, and the *International Journal of Legal Medicine*. A total of 3010 studies that described research on human or animal subjects and/or samples were selected from these journals with only 1079 articles (36%) reporting that they had obtained ethical approval and 527 articles (18%) stating that informed consent was sought either by written or verbal agreement. The authors of this study noted that reported compliance with ethical guidelines in forensic science research and publication was below what is considered minimal reporting rates in biomedical research and encouraged widespread adoption of the 2020 guidelines described below [120].

Guidelines and recommendations for ethnical research on genetics and genomics of biological material were jointly adopted and published in *Forensic Science International: Genetics* [121] and *Forensic Science International: Reports* [122]. These guidelines utilize the following principles as prerequisites for publication in these two journals as well as the *Forensic Science International: Genetics Supplement Series*: (1) general ethics principles that are regulated by national boards and represent widely signed international agreements, (2) universal declarations that require implementations in state members, such as the World Medical Association Declaration of Helsinki biomedical research on human subjects, and (3) universal declarations and principles drafted by independent organizations that have been widely adopted by the scientific community. This includes the U.S. Federal Policy for the Protection of Human Subjects ("Common Rule") that was revised in 2017 (with a compliance date delayed to January 21, 2019).[38]

Submitted manuscripts must provide the following supporting documentation to demonstrate compliance with the publication guidelines: (1) ethical approval in the country of [sample] collection by the appropriate local ethical committee or institutional review board, (2) ethical approval in the country of experimental work according to local legislation; if material collection and experimentation are conducted in different countries, both (1) and (2) are required, (3) template of consent forms in the case of human material as approved by the relevant ethical committee, and (4) approved export/import permits as applicable. Authors must declare in their submitted manuscript that these guidelines have been strictly followed [121,122].

Forensic genetic frequency databases, such as the Y-chromosome Haplotype Reference Database (YHRD), have been challenged over the ethics of DNA holdings, specifically of samples originating from the minority Muslim Uyghur population in western China [123,124]. A survey of U.S. state policies on potential law enforcement access to newborn screening samples found that nearly one-third of states permit these samples or their related data to be disclosed to or used by law enforcement and more than 25% of states have no discernible policy in place regarding law enforcement access [125].

A framework for ethical conduct of forensic scientists as "lived practice" has been proposed, and three case studies were discussed in terms of decision-making processes involving forensic DNA phenotyping and biographical ancestry testing, investigative genetic genealogy, and forensic epigenetics [126]. An ethos for forensic genetics involving the values of integrity, trustworthiness, and effectiveness has likewise been described [127].

#### 2.2.5. Sexual assault kit testing

Unsubmitted or untested sexual assault kits (SAKs) may exist in police or laboratory evidence lockers for many years leading to rape kit backlogs that can spark community outrage when discovered. A number of articles have been published in the past three years describing success rates with examining SAKs and the policies surrounding them. For example, an evaluation of 3422 unsubmitted SAKs in Michigan found 1239 that produced a DNA profile eligible for upload into CODIS with 585 yielding a CODIS hit [128]. In addition, results from a groping and sexual assault case were presented to support the expansion of touch DNA evidence in these types of cases [129].

To assess success rates in their jurisdiction, the Houston Police

[37] See https://www.wmar2news.com/infocus/maryland-quietly-shelves-parts-of-genealogy-privacy-law.

[38] See https://www.hhs.gov/ohrp/regulations-and-policy/regulations/finalized-revisions-common-rule/index.html.

Department randomly selected 491 cases of over 6500 previously unsubmitted sexual assault kits [130]. Of these, 336 cases (68%; 336/491) screened positive for biological evidence; a DNA profile was developed in 270 cases (55%; 270/491) with 213 (43%; 213/491) uploaded to CODIS; and 104 (21% total; 104/491 or 49% of uploaded profiles; 104/213) resulted in a CODIS hit. The statute of limitation had expired in 44% of these CODIS-hit cases, which prohibited arrests and prosecution. Victims were unwilling to participate in a follow-up investigation in another 25% of these cases. When the data were compiled for the publication, charges had been filed in only one CODIS-hit case [130].

Sexual assault cases can be difficult to prosecute as victims may be re-traumatized when a cold case is reopened. The authors of one study shared: "A key to successful pursuit of cold case sexual assaults is to have a well-crafted victim-notification plan and a victim advocate as part of the investigative team" [131]. Interviews with eight assistant district attorneys provided important prosecutors' perspectives on SAK cases, the development of narratives to explain the evidence in a case, and the decision on whether a case should be pursued or what further investigative activities may be needed [132]. The authors concluded: "Our findings suggest that forensic evidence does not magically lead to criminal justice outcomes by itself, but must be used thoughtfully in conjunction with other evidence as part of a well-considered strategy of investigation and prosecution" [132].

Discussing a data set from Denver, Colorado where 1200 sexual assault cold cases with testable DNA samples were examined and 600 cases were processed through the laboratory resulting in 97 CODIS hits, 55 arrests and court filings, and 48 convictions, the authors conclude that the cost of the Denver cold case sexual assault program was worth the investment [131].

From December 2015 to July 2018, the Palm Beach County Sheriff's Office (Florida, USA) researched more than 5500 cases and evaluated evidence from previously untested sexual assault kits spanning a 43-year period at a cost of over $1 million. Of the 1558 sexual assaults examined, there were 686 cases (44%; 686/1558) with CODIS-eligible profiles, 261 CODIS hits, and 5 arrests when the article was written in mid-2019 [133]. The Palm Beach County Sheriff's Office also helped develop a backlog reduction effort through creating a biological processing laboratory within the Boca Raton Police Services Department [134]. With this joint effort from 2016 to 2018, the total average turnaround time decreased from 30 days to under 20 days with the 3489 DNA profiles entered into CODIS resulting in 1254 associations and 965 investigations aided. Important takeaway lessons include the value of (1) engaging legal counsel early to outline necessary legal procedures and the timeline, (2) bringing all stakeholders "to the table" early to discuss expectations, as well as legal and operational responsibilities, and (3) creating a realistic timeline with a comprehensive memorandum of understanding so all parties have agreed to their roles and responsibilities [134].

From 275 previously untested sexual assault kits submitted for DNA testing in one region of Central Brazil, a total of 176 profiles were uploaded to their DNA database resulting in 60 matches (34%; 60/176) and 32 assisted investigations (18%; 32/176) with information about the suspect identity or the connection of serial sexual assaults assigned to the same individual [135]. Another study from the same region of Brazil examined 2165 cases and noted that 13% (286/2165) had information regarding the victim-offender relationship with 63% (179/286) being stranger-perpetrated rapes and 37% (107/286) being non-stranger [136]. The authors then summarize: "Hits were detected only with stranger-perpetrated assaults ($n = 41$), which reinforces that DNA databases are fundamental to investigate sexual crimes. Without DNA typing and DNA databases, probably these cases would never be solved" [136].

Given that laboratories have limited resources and need to prioritize their efforts, some business analytics have been applied to SAK testing. An analysis of the potential societal return on investment (ROI) for processing backlogged, untested SAKs reported a range of 10%–65% ROI depending on the volume of activity for the laboratory conducting the analysis [137]. An evaluation of data from 868 SAKs tested by the San Francisco Policy Department Criminalistics Laboratory during 2017–2019 found that machine learning algorithms outperformed forensic examiners in flagging potentially probative samples [138].

An examination of 5165 SAKs collected in Cuyahoga County (Ohio, USA) from 1993 through 2011 found 3099 with DNA of which 2127 produced a CODIS hit, with 803 investigations leading to an indictment and eventually 78 to trial along with 330 pleas [139]. The authors report a "cost savings to the community of $26.48 million after the inclusion of tangible and intangible costs of future sexual assaults averted through convictions" and advocate for "the cost-effectiveness of investigating no CODIS hit cases and support an 'investigate all' approach" [139]. Likewise an assessment of 900 previously-untested SAKs from Detroit (Michigan, USA) found that "few of the tested variables were significant predictors of CODIS hit rate" and "testing all previously-unsubmitted kits may generate information that is useful to the criminal justice system, while also potentially addressing the institutional betrayal victims experienced when their kits were ignored" [140].

A group in the Philippines described an integrated system to improve their SAK processing [141]. With an optimized workflow in Montreal, Canada, SAK processing median turnaround time decreased from 140 days to 45 days with a foreign DNA profile being obtained in 44% of cases [142]. In addition, this group examined casework data to guide resource allocation through identifying the likelihood of specific types of cases and samples yielding foreign biological material [142]. Decision trees and logistic regression models were also used to try and predict whether or not SAKs will yield a CODIS-eligible DNA profile [143]. Finally, direct PCR and rapid DNA approaches to streamline SAK testing were reviewed [144].

### 2.3. Forensic biology and body fluid identification

The basic workflow for biological samples in forensic examinations typically involves a visual examination of the evidence, a presumptive and/or confirmatory test for a suspected body fluid (e.g., the amylase assay for saliva), and DNA analysis and interpretation [145]. Body fluid identification (BFID), in particular with blood, saliva, semen, or vaginal fluid stains, provides valuable evidence in many investigations that can aid in the resolution of a crime [146]. Many of these BFID tests are presumptive and not nearly as sensitive as modern DNA tests meaning that "obtaining a DNA profile without being able to associate [it] with a body fluid is an increasingly regular occurrence" and "it is necessary and important, especially in the eyes of the law, to be able to say which body fluid that the DNA profile was obtained from" [147].

A number of approaches are being taken to improve the sensitivity and specificity of BFID in recent years including DNA methylation [148–161], messenger RNA (mRNA) [162–166], microRNA (miRNA) [167], protein mass spectrometry for seminal fluid detection [168], and microbiome analysis [169,170]. Although many new techniques are being described in the scientific literature, traditional methods for semen identification are still widely used in regular forensic casework [171].

When using RNA assays, DNA and RNA are co-extracted from examined samples [172,173]. Some tests may only distinguish between two possible body fluids, such as saliva and vaginal fluid [174], while other tests may attempt to distinguish six forensically relevant body fluids – vaginal fluid, seminal fluids, sperm cells, saliva, menstrual blood, and peripheral blood – although not always as clearly as desired [175]. BFID assays must also cope with mixed body fluids [176].

### 2.4. DNA collection and extraction

The process of obtaining a DNA profile begins with collecting a biological sample and extracting DNA from it. A review of recent trends

and developments in forensic DNA extraction focused on isolating male DNA in sexual assault cases, using portable rapid DNA testing instruments, recovering DNA from difficult samples such as human remains, and bypassing DNA extraction altogether with direct PCR methods [177].

#### 2.4.1. Touch evidence and fingerprint processing methods

Various studies have explored the compatibility of common fingerprint processing methods with DNA typing results [178–188]. For example, DNA recovery was explored after various steps in three different latent fingerprint processing methods – and fewer treatments were judged preferable with a 1,2-indanedione-zinc (IND/Zn) method appearing least harmful to downstream DNA analysis [187]. A different study found improved recovery of DNA from cigarette butts following latent fingerprint processing with 1,8-diazafluoren-9-one (DFO) compared to IND/Zn [179].

DNA losses were quantified with mock fingerprints deposited on four different surfaces to better understand DNA collection and extraction method performance [189]. The application of Diamond Dye has been shown to enable visualization of cells deposited on surfaces without interfering with subsequent PCR amplification and DNA typing [190–192].

It was possible to recover DNA profiles from clothing that someone touched for as little as 2 s [193]. DNA sampling success rates from car seats and steering wheels were studied [194] and recovery of DNA from vehicle surfaces using different swabs was explored [195]. In addition, the double-swab technique, where a wipe using a wet swab is followed by a wipe with a dry one, was revisited with an observation that for non-absorbing surfaces, the first web swab yielded 16 times more DNA than the second dry swab [196]. Swabs of cotton, flocked nylon, and foam reportedly provided equivalent DNA recoveries for smooth/non-absorbing surfaces, and an optimized swabbing technique involving the application of a 60-degree angle and rotating the swab during sampling improved DNA yields for cotton swabs [197].

#### 2.4.2. Results from unfired and fired cartridge cases

Ammunition needs to be handled to load a weapon and thus DNA from the handler may be deposited onto the ammunition via touch [198]. Important progress has been made in recovering DNA from ammunition such as unfired cartridges or fired cartridge cases (FCCs) that may remain at a crime scene after a weapon has been fired. Trace quantities of DNA recovered from firearm or FCC surfaces has been used to try and link results to gun-related crimes.

A 2019 review of the literature regarding obtaining successful DNA results from ammunition examined collection techniques, extraction methodologies, and various amplification kits and conditions [199]. A direct PCR approach detected more STR alleles than methods using DNA extraction, and the authors noted that mixtures are commonly observed from gun surfaces, bullets, and cartridges in both controlled experimental conditions and from actual casework evidence and they encourage careful interpretation of these results [200]. The development of a crime scene FCC collector was combined with a new DNA recovery method that uses a rinse-and-swab technique [201].

Research studies and review articles have considered factors affecting DNA recovery from cartridge cases and the impact of metal surfaces on DNA recovery [202–209]. Recovery of mtDNA from unfired ammunition components has been assessed for sequence quality [210].

### 2.5. DNA typing

Following collection of DNA evidence and its extraction from biological samples, the typical typing process involves DNA quantitation, PCR amplification of STR markers, and STR typing using capillary electrophoresis. Direct PCR avoids the DNA extraction and quantitation steps, which can improve recovery of trace amounts of DNA [211,212]. Whole genome amplification prior to STR analysis has also been examined to aid recovery of degraded DNA [213] and to enable profiling of single sperm cells [214].

PCR amplification using STR typing kits can sometimes produce artifacts that impact DNA interpretation including missing (null) alleles [215], false tri-allelic patterns [216] or extra peaks when amplified in the presence of microbial DNA [217–219].

Applied Biosystems Genetic Analyzers have been the primary means of performing multi-colored capillary electrophoresis for many years [4]. First experiences with Promega's new Spectrum Compact CE System have recently been reported [220]. A number of new research and commercial STR kits have been introduced in recent years along with the publication of at least 24 validation studies (Table 5). These validation studies typically follow guidelines outlined by the ENFSI DNA Working Group,[39] SWGDAM[40], or a 2009 Chinese National Standard.[41]

A report on the first two years of submissions to the STRidER[42] (STRs for Identity ENFSI Reference) database for online allele frequencies revealed that 96% of the submitted 165 autosomal STR datasets generated by CE contained errors, showing the value of centralized quality control and data curation [245].

### 2.6. DNA interpretation at the source or sub-source level

The designation of STR alleles and genotypes of contributors in DNA mixtures are key aspects of DNA interpretation [246,247]. Electropherograms generated by CE instruments exhibit both STR alleles and artifacts that complicate data interpretation. Efforts are underway to understand and model instrumental artifacts [248–251] as well as biological artifacts of the PCR amplification process such as STR stutter products [252,253]. Machine learning approaches are being applied to classify artifacts versus alleles with the goal to eventually replace manual data interpretation with computer algorithms [254–257]. One such program, FaSTR DNA, enables potential artifact peaks from stutter, pull-up, and spikes to be filtered or flagged, and a developmental validation has been published examining 3403 profiles generated with seven different STR kits [258].

#### 2.6.1. DNA mixture interpretation

Forensic evidence routinely contains contributions from multiple donors, which result in DNA mixtures. A number of approaches have been taken and advances made in DNA mixture interpretation [259]. These include probabilistic genotyping software [15], using genetic markers beyond traditional autosomal STR typing [260], or separating contributor cells and performing single-cell analysis [261–266].

In June 2021, the National Institute of Standards and Technology (NIST) released a draft report regarding the scientific foundations of DNA mixture interpretation [267]. This 250-page document described 16 principles that underpin DNA mixture interpretation, provided 25 key takeaways, and cited 528 references. NIST also began a Human Factors Expert Working Group on DNA Interpretation in February 2020 and plans to release a report with recommendations in 2023.

Assessment of the number of contributors (NoC) is a critical element of accurate DNA mixture interpretation. For example, the LRs relating to minor contributors can be reduced when the incorrect number of contributors is assumed [268]. Allele sharing among contributors to a mixture and masking of alleles due to STR stutter artifacts can lead to inaccurate NoC estimates based on simply counting the number of alleles at a locus. Different approaches and software programs have been used for NoC estimation [269–275]. Total allele count (TAC) distribution via TAC curves showed an improvement in manually estimating the number of contributors with complex mixtures [276]. Sequence analysis

[39] See https://enfsi.eu/about-enfsi/structure/working-groups/dna/.
[40] See https://www.swgdam.org/publications.
[41] See https://www.chinesestandard.net/PDF/English.aspx/GAT815-2009.
[42] See https://strider.online/.

**Table 5**
STR kits assessed with 24 published validation studies during 2019–2022.

| Publication | STR Kit/Primer Set | Comments |
|---|---|---|
| Al Janaahi et al., 2019 [221] | VeriFiler Plus | Validation studies (sensitivity, peak height ratio, precision, reproducibility, thresholds, mixtures, concordance) |
| Alsafiah et al., 2019 [222] | SureID 23comp Human Identification | Validation studies (following ENFSI and SWGDAM guidelines); has 17 non-CODIS STRs |
| Bai et al., 2019 [223] | DNATyper25 | Validation studies (following SWGDAM and China National Standard); has 20 non-CODIS STRs |
| Cho et al., 2021 [224] | Investigator 24plex QS, PowerPlex Fusion, GlobalFiler | Examined 189 casework samples and compared performance across the three kits |
| Fan et al., 2021 [225] | STRtyper-32G | Developmental validation studies (SWGDAM); has 10 non-CODIS STRs |
| Green et al., 2021 [226] | VeriFiler Plus | Developmental validation studies (SWGDAM); concordance checked with Huaxia Platinum kits |
| Hakim et al., 2020 [227] | Investigator 24plex GO! | Validation studies; concordance with GlobalFiler |
| Harrel et al., 2021 [228] | Investigator 24plex QS and GO! | Assessment of sample quality metrics in both kits |
| Jiang et al., 2021a [229] | STRscan-17LC kit | Validation studies (SWGDAM) |
| Jiang et al., 2021b [230] | Novel 8-dye STR multiplex | Validation studies (SWGDAM); 18 STRs plus AMEL; detection with GA118-24B Genetic Analyzer |
| Lenz et al., 2020 [231] | VersaPlex 27PY system | Developmental validation studies (SWGDAM); includes D6S1043 |
| Li et al., 2021 [232] | SureID S6 system | Validation studies (SWGDAM); concordance with Huaxia Platinum kit; uses lyophilized reagents |
| Liu et al., 2019 [233] | 19 autosomal and 27 Y-STRs | Validation studies (Chinese National Standard); 47 loci (Fusion 6C, GlobalFiler, Yfiler Plus) with 6-dyes |
| Qu et al., 2019 [234] | Microreader 20A ID system | Developmental validation studies (SWGDAM) |
| Qu et al., 2021 [235] | Novel 6-dye, 31-plex | Developmental validation studies (SWGDAM and Chinese National Standard); 29 STRs, AMEL, Y-InDel |
| Wang et al., 2020a [236] | 21plex with DYS391 and ABO | Describes a 21plex with 18 autosomal STRs, ABO blood group locus, DYS391, and AMEL |
| Wang et al., 2020b [237] | Investigator 26plex QS kit | Validation studies (SWGDAM); concordance with AGCU Expressmarker 22 kit |
| Xie et al., 2020 [238] | AGCU Expressmarker 16 + 22Y | Developmental validation studies (SWGDAM) |
| Xie et al., 2022 [239] | Novel 26plex | Validation studies (SWGDAM); multiple STRs on chromosomes 13, 18, 21, and X for prenatal diagnosis |
| Yin et al., 2021 [240] | Microreader 28A ID System | Developmental validation (SWGDAM); concordance with AGCU Expressmarker 22 kit |
| Zhang et al., 2020 [241] | SiFaSTR 21plex_NCII | Developmental validation (SWGDAM); describes 18 new non-CODIS STR loci |
| Zhang et al., 2021 [242] | AGCU Expressmarker 30 Kit | Developmental validation (SWGDAM); includes 6 non-CODIS STR loci; concordance with AGCU Expressmarker 22 kit |
| Zheng et al., 2019 [243] | SiFaSTR 23-plex panel | Developmental validation (SWGDAM and Chinese National Standard) |
| Zhong et al., 2019 [244] | Huaxia Platinum PCR kit | Developmental validation (SWGDAM and Chinese National Standards) |

of STR loci expands the number of possible alleles compared to CE-based length measurements and thus can improve NoC estimates [277].

In the past three years, validation studies have been performed with a number of probabilistic genotyping software (PGS) systems including EuroForMix [278], DNAStatistX [279,280], TrueAllele [281], STRmix [282], Statistefix [283], Mixture Solution [284], Kongoh [285], and MaSTR [286,287]. Developers of EuroForMix, DNAStatistX, and STRmix provided a review of these systems [288]. Multi-laboratory assessments have been described [289,290] and likelihood ratios obtained from EuroForMix and STRmix compared [291–294]. With a growing literature in this area, there are many other articles that could have been cited.

### 2.7. DNA interpretation at the activity level

DNA interpretation at the source or sub-source level helps to answer the question of *who* deposited the cell material, whether attribution for the result can be made to a specific cell type (i.e., source level) or simply to the DNA if no attribution can be made to a specific cell type (i.e., sub-source level). Activity-level propositions seek to answer the question of *how* did an individual's cell material get there. Interpretation at the activity level is sometimes referred to as evaluative reporting [295,296].

In 2020, the ISFG DNA Commission [38] discussed the why, when, and how to carry out evaluative reporting given activity level propositions through providing examples of formulating these propositions. These Commission recommendations emphasize that reports using a likelihood ratio based on case-specific propositions and relevant conditioning information should highlight the assumptions being made and that "it is not valid to carry over a likelihood ratio from a low level, such as sub-source, to a higher level such as source or activity propositions … because the LRs given sub-source level propositions are often very high and LRs given activity level propositions will often be many orders of magnitude lower" [38]. Another recommendation specifies that "scientists must not give their opinion on what is the 'most likely way of transfer' (direct or indirect), as this would amount to giving an opinion on the activities and result in a prosecutor's fallacy (i.e., give the probability *that* X is true). The scientists' role is to assess the value of the *results* if each proposition is true in accordance with the likelihood ratio framework (the probability of the *results if* X is true and *if* Y is true)" [38] (emphasis in the original). This DNA Commission provided 11 recommendations and 4 considerations that should be studied carefully by those who implement activity-level DNA interpretation.

#### 2.7.1. DNA transfer and persistence studies

To evaluate DNA findings given activity-level propositions it is important to understand the factors and variables that may impact DNA transfer, persistence, prevalence, and recovery (DNA-TPPR). These factors include history of contacting surfaces, biological material type, quantity and quality of DNA, dryness of biological material, manner and duration of contact, number and order of contacts, substrate type(s), time lapses and environment, and methods and thresholds used in the forensic DNA laboratory to generate the available data [297].

Three valuable review articles were published on this topic in 2019 [14,28,299]. Following a comprehensive January 2019 review that cited [298] references on DNA-TPPR [14], the same authors provided an update in November 2021 on recent progress towards meeting challenges and a synopsis of 144 relevant articles published between January 2018 and March 2021 [297]. While few studies provide the information needed to help assign probabilities of obtaining DNA results given specific sets of circumstances, progress includes use of Bayesian Networks [300] to identify variables for complex transfer scenarios [38, 301–305] as well as development of an online database DNA-TrAC[43] for relevant research articles [299] and a structured knowledge base[44] with information to help practitioners interpret general transfer events at an activity level [306].

[43] See https://bit.ly/2R4bFgL (DNA-TrAC).
[44] See https://cieqfmweb.uqtr.ca/fmi/webd/OD_CIEQ_CRIMINALISTIQUE (Transfer Traces Activity DataBase).

Forensic DNA pioneer Peter Gill emphasized that awareness of the limitations of DNA evidence is important for users of this data given that an increased sensitivity of modern DNA methods means that DNA may be recovered that is irrelevant to the crime under investigation [307]. An ISFG DNA Commission (see Section 1.2.5) emphasized that the strength of evidence associated with a DNA match at the sub-source level cannot be carried over to activity level propositions [38]. Structuring case details into propositions, assumptions, and undisputed case information has been encouraged [308].

Factors affecting variability of DNA recovery on firearms were studied with four realistic, casework-relevant handling scenarios along with results obtained including DNA quantities, number of contributors, and relative profile contributions for known and unknown contributors [309]. These studies found that sampling several smaller surfaces on a firearm and including the sampling location in the evaluation process can be helpful in assessing results given alternative activity-level propositions in gun-related crimes. The authors recommend that "further extensive, detailed and systematic DNA transfer studies are needed to acquire the knowledge required for reliable activity-level evaluations" [309].

Other recent studies on DNA-TPPR include examining prevalence and persistence of DNA or saliva from car drivers and passengers [310–312], evaluation of DNA from regularly-used knives after a brief use by someone else [313], studying the accumulation of endogenous and exogenous DNA on hands [314] and non-self-DNA on the neck [315], considering the potential of DNA transfer via work gloves [316, 317] or during lock picking [318], and investigating whether DNA can be recovered from illicit drug capsules [319,320] or packaging [321] to identify those individuals preparing or handling the drugs.

Efforts have been made to estimate the quantity of DNA transferred in primary versus secondary transfer scenarios [322]. As quantities of DNA transferred can be highly variable and thought to be dependent on the so-called "shedder status" – how much DNA an individual exudes, several studies explored this topic [323–327]. Studies have also considered the level of DNA an individual transfers to untouched items in their immediate surroundings [328], the position and level of DNA transferred during digital sexual assault [329] or during various activities with worn upper garments [330,331], and the DNA composition on the surface of evidence bags pre- and post-exhibit examination [332]. Studies assessing background levels of male DNA on underpants worn by females [333] and background levels of DNA on flooring within houses [334] are providing important knowledge about the possibilities and probabilities of DNA transfer and persistence.

The authors of one study summarize some key points that could be extended to many other studies as words of caution: "From a wider trace DNA point of view, this study has demonstrated that the person who most recently handled an item may not be the major contributor and someone who handled an item for longer may still not be the major contributor if they remove more DNA than they deposit. The amount of DNA transferred and retained on an item is highly variable between individuals and even within the same individual between replicates" [320].

## 3. Emerging technologies, research studies, and other topics

New technologies to aid forensic DNA typing are constantly under development. This section explores recent activities with next-generation DNA sequencing, DNA phenotyping for estimating a sample donor's age, ancestry, and appearance, lineage markers, other markers and approaches, and non-human DNA and wildlife forensics, and is expected to be of value to researchers and those practitioners looking to future directions in the field.

### 3.1. Next-generation sequencing

Next-generation sequencing (NGS), also known as massively parallel sequencing (MPS) in the forensic DNA community, expands the measurement capabilities and information content of a DNA sample beyond the traditional length-based results with STR markers obtained with capillary electrophoresis (CE) methods. Additional genetic markers, such as single nucleotide polymorphisms (SNPs), microhaplotypes, and mitochondrial genome (mtGenome) sequence, may be analyzed along with the full sequence of STR alleles. This higher information content per sample opens up new potential applications such as phenotyping of externally visible characteristics and biogeographical ancestry as described in review articles [335,336].

As mentioned in Section 1.2.1, the NIJ Forensic Laboratory Needs Technology Working Group (FLN-TWG) published a 29-page implementation strategy on next-generation sequencing for DNA analysis in September 2021 [28]. This guide discusses how NGS works and its advantages and disadvantages, the various instrument platforms and commercial kits available with approximate costs, items to consider regarding facilities, data storage, and personnel training, and resources for implementing NGS technology. A total of 73% of 105 forensic DNA laboratories surveyed from 32 European countries already own an MPS platform or plan to acquire one in the next year or two and one-third of the survey participants already conduct MPS-based STR sequencing, identity, or ancestry SNP typing [337].

Validation studies have been described with the ForenSeq DNA Signature Prep kit and the MiSeq FGx system [338–340], with the Verogen ForenSeq Primer Mix B for phenotyping and biogeographical ancestry predictions [341,342], and for resizing reaction volumes with the ForenSeq DNA Signature Prep kit library preparation [343]. MPS sequence data showed excellent allele concordance with CE results for 31 autosomal STRs in the Precision ID GlobalFiler NGS STR Panel from 496 Spanish individuals [344] and from 22 autosomal STR loci in the PowerSeq 46GY panel with 247 Austrians [345].

STR flanking region sequence variation has been explored [346] and reports of population data and sequence variation were published for samples from India [347], France [348], China [349,350], Korea [351], Brazil [352], Tibet [353], and the United States [354].

In April 2019 the STRAND (Short Tandem Repeat: Align, Name, Define) Working Group was formalized [355] to consider several possible approaches to sequence-based STR nomenclature that have been proposed [356,357]. An overview of software options has been provided for analysis of forensic sequencing data [358]. Some recent published options include STRinNGS [359], STRait Razor [360], ArmedXpert tools MixtureAce and Mixture Interpretation to analyze MPS-STR data [361], and STRsearch for targeted profiling of STRs in MPS data [362]. To aid interpretation of MPS-STR data, sensitivity studies were performed with single-source samples and sequence data analyzed by DNA quantity and method used [363]. A procedure has been described to address calculation of match probabilities when results are generated using MPS kits with different trim sites than those present in the relevant population frequency database [364]. Performance of different MPS kits, markers, or methods can be compared for accuracy and precision using the Levenshtein distance metric [365].

Novel MPS STR and SNP panels developed in recent years include IdPrism [366], a QIAGEN 140-locus SNP panel [367], the 21plex monSTR identity panel [368], a 42plex STR NGS panel to assist with kinship analysis [369], the 5422 marker FORCE (FORensic Capture Enrichment) panel [370], a forensic panel with 186 SNPs and 123 STRs [371], the SifaMPS panel for targeting 87 STRs and 294 SNPs [372], a 1245 SNP panel [373], 90 STRs and 100 SNPs for application with kinship cases [374], an adaption of the SNPforID 52plex panel to MPS [375], 448plex SNP panel [376], a 133plex panel with 52 autosomal and 81 Y-chromosome STRs [377], and a forensic identification multiplex with 1270 tri-allelic SNPs involving 1241 autosomal and 29 X-chromosome markers [378]. The 124 SNPs in the Precision ID Identity Panel were examined in a central Indian population [379] and human leukocyte antigen (HLA) alleles used in the early 1990s were revisited with MPS capability [380–382].

MPS methods have demonstrated utility with compromised samples [383–388] and mixture interpretation [389–395]. Microhaplotype assays have also been developed to assist with DNA mixture deconvolution [396,397]. Collaborative studies have explored variability with laboratory performance using MPS methods [398,399]. Population structure [400] and linkage and linkage disequilibrium [401] were examined among the markers in forensic MPS panels.

A review of transcriptome analysis using MPS discussed efforts with body fluid and tissue identification, determination of the time since deposition of stains and the age of donors, the estimation of post-mortem interval, and assistance to post-mortem death investigations [402]. The potential for MPS methods to assist with environmental trace analysis was reviewed in terms of forensic soil analysis, forensic botany, and human identification utilizing the skin microbiome [403]. The possibility of non-invasive prenatal paternity testing using cell-free fetal DNA from maternal plasma was explored with the Precision ID Identity Panel [404] and the ForenSeq DNA Signature Prep Kit [405]. Pairwise kinship analysis was also examined using the ForenSeq DNA Signature Prep Kit and multi-generational family pedigrees [406,407]. Nanopore sequencing has also been explored for sequencing STR and SNP markers [408–416].

### 3.2. DNA phenotyping (ancestry, appearance, age)

Continuing research into the genetic components of biogeographic ancestry, appearance, and age predictions have improved forensic DNA phenotyping capabilities [417]. These forensic innovations may sometimes impact public expectations [418]. The investigation in a murder case was assisted using information from forensic DNA phenotyping that predicted eye, hair, and skin color of an unknown suspect with the HIrisPlex-S system involving targeted massively parallel sequencing [419].

The VISAGE (Visible Attributes Through Genomics) Consortium, which consists of 13 partners from academic, police, and justice institutions in 8 European countries, has established new scientific knowledge and developed and tested prototype tools for DNA analysis and statistical interpretation as well as conducted education for stakeholders. In the 2019 to 2022 time window of this review, this concerted effort produced[45] one review article [417], 22 original research publications [337,420–440], and three reports [441–443].

DNA phenotyping is currently an active area of research, and numerous activities and publications exist beyond the VISAGE articles noted here. Another 137 articles have appeared in the literature in the past three years on biogeographical ancestry, appearance (primarily hair color, eye color, and skin color), and biological age predictions (typically utilizing DNA methylation) (see **Supplemental File**).

### 3.3. Lineage markers (Y-chromosome, mtDNA, X-chromosome)

Lineage markers consist of Y-chromosome, mitochondrial DNA, and X-chromosome genetic information that may be inherited from just one parent without the regular recombination that occurs with autosomal DNA markers. Research in terms of new markers, assays, and population studies continue to be published for these lineage markers.

#### 3.3.1. Y-chromosome

Several recent review articles were published on forensic applications of Y-chromosome testing [444–446]. As discussed previously in Section 1.2, an ISFG DNA Commission summarized the state of the field with Y-STR interpretation [39]. Rapidly mutating Y-STR loci can be used to differentiate closely related males [447–449]. New statistical approaches to assessing evidence with Y-chromosome information have been described [450,451]. Four commercial Y-STR multiplexes were compared with the NIST 1032 U S. population sample set and the allele and haplotype diversities explored with length-based versus sequence-based information [452].

A number of Y-STR typing systems have been described along with validation studies, such as a 36plex [453], a 41plex [454], a 29plex [455], a 17plex [456], a 24plex [457], the Microreader 40Y ID System [458], the 24 Y-STRs in the AGCU Y SUPP STR kit [459], the DNATyper Y26 PCR amplification kit [460], a multiplex with 12 multicopy Y-STR loci [461], the Yfiler Platinum PCR Amplification Kit [462], a 45plex [463], the Microreader 29Y Prime ID system [464], an assay with 30 slow and moderate mutation Y-STR markers [465], the 17plex Microreader RM-Y ID System [466], and a 26plex for rapidly mutating Y-STRs [467]. A machine learning program predicted Y haplogroups using two Y-STR multiplexes with 32 Y-STRs [468].

Deletions and duplications with 42 Y-STR were reported in a sample of 1420 unrelated males and 1160 father-son pairs from a Chinese Han population [469]. Using Y-STR allele sequences has enabled locating parallel mutations in deep-rooting family pedigrees [470]. The surname match frequency with Y-chromosome haplotypes was explored using 2401 males genotyped for 46 Y-STRs and 183 Y-SNPs [471]. In the Y-chromosome's role as a valuable kinship indicator to assist in genetic genealogy and forensic research, models to improve prediction of the time to the most recent common paternal ancestor have been studied with 46 Y-STRs and 1120 biologically related genealogical pairs [472]. A massively parallel sequencing tool was developed to analyze 859 Y-SNPs to infer 640 Y haplogroups [473]. Another MPS tool, the CSYseq panel, targeted 15,611 Y-SNPs to categorize 1443 Y-sub-haplogroup lineages worldwide along with 202 Y-STRs including 81 slow, 68 moderate, 27 fast, and 26 rapidly mutating Y-STRs to individualize close paternal relatives [474].

#### 3.3.2. Mitochondrial DNA

Mitochondrial DNA (mtDNA), which is maternally inherited with a high copy number per cell, can aid human identification, missing persons investigations, and challenging forensic specimens containing low quantities of nuclear DNA such as hair shafts [475–477]. Validation studies have been published using traditional Sanger sequencing [478] and next-generation sequencing [479–481]. Illumina and Thermo Fisher now provide mtDNA whole genome NGS assays [482–485]. Many mtDNA population data sets were published in the past three years including high-quality data from U.S. populations [486]. The suitability of current mtDNA interpretation guidelines for whole mtDNA genome (mtGenome) comparisons has been evaluated [487].

NGS methods have increased sensitivity of mtDNA heteroplasmy detection [488,489], which can influence the ability to connect buccal reference samples and rootless hairs from the same individual [490,491]. Twelve polymerases were compared in terms of mtDNA amplification yields from challenging hairs – with KAPA HiFi HotStart and PrimeSTR HS outperforming AmpliTaq Gold DNA polymerase that is widely used in forensic laboratories [492]. Multiple studies and review articles have discussed distinguishing mtDNA from nuclear DNA elements of mtDNA (NUMTs) that have been inserted into our nuclear DNA [493–496].

NGS sequencing of the mtGenome has permitted improved resolution of the most common West Eurasian mtDNA control region haplotype [497]. Phylogenetic alignment and haplogroup classification have continued to be refined with new sequence information [498], and new assays have been developed to aid haplogroup classification [499]. Concerns over potential paternal inheritance of mtDNA have also been addressed [500,501].

#### 3.3.3. X-chromosome

A 20-year review of X-chromosome use in forensic genetics examined the number and types of markers available, an overview of worldwide population data, the use of X-chromosome markers in complex kinship testing, mutation studies, current weaknesses, and future prospects

[45] See https://www.visage-h2020.eu/index.html#publications.

[502]. One example of the forensic application of X-chromosome markers include use in relationship testing cases involving suspicion of incest or paternity without a maternal sample for comparison [503]. Four new X-STR multiplex assays were described along with validation studies including a 19plex [504], a 16plex [505], another 19plex – the Microreader 19X Direct ID System [506], and an 18plex named TYPER-X19 multiplex assay [507]. A collaborative study examined paternal and maternal mutations in X-STR markers [508]. A software program for performing population statistics on X-STR data was introduced [509] and sequence-based U.S. population data described for 7 X-STR loci [510].

### 3.4. New markers and approaches (microhaplotypes, InDels, proteomics, human microbiome)

In this section on new markers and approaches, publications related to microhaplotypes and insertion/deletion (InDel, or DIP for deletion insertion polymorphisms) markers are reviewed along with proteomic and microbiome approaches to supplement standard human DNA typing methods.

#### 3.4.1. Microhaplotypes

Microhaplotype (MH) markers consist of multiple SNPs in close proximity (e.g., typically <200 bp or <300 bp) that can be simultaneously genotyped with each DNA sequence read using NGS. Two or more linked SNPs will define three or more haplotypes. Compared to STR markers, MHs do not have stutter artifacts (which complicate mixture interpretation), can be designed with shorter amplicon lengths in some cases (which benefits recovery of genetic information from degraded DNA samples), possess a higher degree of polymorphism compared to single SNP loci (which benefits discrimination power), and exhibit low mutation rates (which enables relationship testing and biogeographical ancestry inference). Thus, MH markers bring advantages to human identification, ancestry inference, kinship analysis, and mixture deconvolution to potentially assist missing person investigations, relationship testing, and forensic casework as discussed in several recent reviews [16,511]. A new database, MicroHapDB, has compiled information on over 400 published MH markers and frequency data from 26 global population groups [512].

A number of MH panels have been described [513–519]. Population data has been collected from a number of sources around the world including four U.S. population groups examined with a 74plex assay with 74 MH loci and 230 SNPs [520]. Various MH panels have been evaluated for effectiveness with kinship analysis [521–523]. Likewise the ability to detect minor contributors in DNA mixtures has been assessed [524–526].

#### 3.4.2. InDel markers

InDel markers can be detected using a CE-based length analysis, and thus use instrumentation that forensic DNA laboratories already have. InDels can also be designed to amplify short DNA fragments (e.g., <125 bp) to help improve amplification success rates with low DNA quantity and/or quality. However, with only two possible alleles like SNPs, InDels are not as polymorphic as STRs and thus require more markers to obtain similar powers of discrimination as multi-allelic STR markers and do not work as well with mixed DNA samples. InDels possess a lower mutation rate than STRs and can be used as ancestry informative markers (AIMs) since allele frequencies may differ among geographically separated population groups.

Two commercial InDel kit exist: (1) Investigator DIPlex (QIAGEN, Hilden, Germany) with 30 InDels [527–531] and (2) InnoTyper 21 (InnoGenomics, New Orleans, Louisiana, USA) with 21 autosomal insertion-null (INNUL) markers [532–535]. In addition, a number of InDel assays have been published including a 32plex [536], a 35plex [537], a 38plex [538], a 39plex with AIMs [539], a 43plex [540], a 57plex [541], a 60plex with 57 autosomal InDels, 2 Y-chromosome InDels, and amelogenin [542], a 32plex with X-chromosome InDels [543], and a 21plex with AIMs [544].

A multi-InDel marker is a specific DNA fragment with more than one InDel marker located tightly in the physical position that provides a microhaplotype [545]. Several multi-InDel assays have been published include a 12plex [546] and an 18plex [547].

#### 3.4.3. Proteomics

Protein analysis, often through immunological assays, has traditionally been used to identify body fluids and tissues. With improvements in protein mass spectrometry in recent years, genetic variation can be observed in hair shafts via single amino acid polymorphisms. Detection of these genetically variant peptides (GVPs) can infer the presence of corresponding SNP alleles in the genome of the individual who is the source of the protein sample. A thorough review of forensic proteomics in 2021 cited 375 references [18]. Recent efforts in this area have focused on using GVPs to differentiate individuals through their human skin cells [548–550] or hair samples [551–559]. An algorithm has been proposed for calculating random match probabilities with GVP information [560].

#### 3.4.4. Human microbiome

Microorganisms live in and on the human body, and efforts are underway to utilize the human microbiome for a variety of potential forensic applications [21,561–563]. There are also active efforts with analysis of microbiomes in the environment (e.g., soil or water samples), which could be classified under non-human DNA testing. Forensic microbiome research covers at least six areas: (1) individual identification, (2) tissue/body fluid identification, (3) geolocation, (4) time since stain deposition estimation, (5) forensic medicine, and (6) post-mortem interval (PMI) estimation. Biological, technical, and data issues have been raised and potential solutions explored in a recent review article [21]. For example, microbes on deceased individuals are being studied to estimate the postmortem interval [20] and postmortem skin microbiomes were found to be stable during repeated sampling up to 60 h postmortem [564].

Sequence analysis of 16S rRNA using NGS provides information on the microbiome community present in a tested sample [565]. The Forensic Microbiome Database[46] correlates publicly available 16S rRNA sequence data as a community resource. If the skin microbiome is extremely diverse among individuals, then the potential exists to associate the bacterial communities on an individual's skin with objects touched by this individual assuming that the bacteria originating from the donor's skin are deposited (i.e., transfer to and persist on the surface) and can be detected and interpreted.

Specific aspects of the microbiome (e.g., the bacterial community) may be able to provide details about the donor through bacterial profiling. For example, in one study correlations were observed between the bacterial profile and gender, ethnicity, diet type, and hand sanitizer used [566]. Another study with 30 individuals found that each person left behind microbial signatures that could be used to track interaction with various surfaces within a building, but the authors concluded "we believe the human microbiome, while having some potential value as a trace evidence marker for forensic analysis, is currently under-developed and unable to provide the level of security, specificity and accuracy required for a forensic tool" [565].

Direct and indirect transfer of microbiomes between individuals has been studied [567,568] along with identifying background microbiomes [569] and the possibility of transfer of microbiomes within a forensic laboratory setting [570]. Changes in four bacterial species in saliva stains were charted, showing that it was possible to correctly predict deposition time within one week in 80% of the stains [571]. The ability to detect sexual contact has been explored through using the

[46] See http://fmd.jcvi.org/.

microbiome of the pubic region [572–574]. The microbiomes on skin, saliva, vaginal fluid, and stool samples have been compared [575]. The stability, diversity, and individualization of the human skin virome was explored with 59 viral biomarkers being found that differed across the 42 individuals studied [576]. It will be interesting to see what the future holds and what other findings come from this active area of research.

### 3.5. Kinship analysis, human identification, and disaster victim identification

Kinship analysis, which uses genetic markers and statistics to evaluate the potential for specific biological relationships, is important for parentage testing, disaster victim identification (DVI), and human identification of remains that may be recovered in missing person cases. New open-source software programs have been described that can assist with kinship analysis [577,578].

A potential biological relationship is commonly evaluated using a likelihood ratio (LR) by comparing the likelihoods of observing the genetic data given two alternative hypotheses, such as (1) an individual is related to another individual in a defined relationship versus (2) the two individuals not related. Higher LR values indicate stronger support with the genetic data if the proposed relationship is true. Multiple factors influence LR kinship calculations including the specific hypotheses, the genetic markers examined, the allele frequencies of the relevant population(s), the co-ancestry coefficient applied, and approaches to address potential mutations. STR genotypes were reported for 11 population groups used by the FBI Laboratory [579]. The status quo has been challenged in recent articles regarding how hypotheses are commonly established [580] and whether race-specific U.S. population databases should be used for allele frequency calculations [581].

Depending on the relationship being explored, information can be optimized through genetic information from additional known relatives or through collecting results at more loci [582]. Potential error rates have been modeled with the observation that false negatives, which occur when related individuals are misinterpreted as being unrelated, are more common than false positives, where unrelated people are interpreted as being related [583]. While LRs are generally reliable in detecting or confirming parent/child pairs, limitations of kinship determinations exist (e.g., distinguishing siblings from half-siblings) when using STR data [584].

Pairwise comparisons have been studied in forensic kinship analysis [585–587]. The effectiveness of 40 STRs plus 91 SNPs was shown to be better than 27 STRs and 91 SNPs or 40 STRs alone [588]. Only a minor increase in LRs was observed when taking NGS-generated allele sequence variation rather than fragment length allele variation [589]. The statistical power of exclusion and inclusion can be used to prioritize family members selected for testing in resolving missing person cases [590]. A strategy for making decisions when facing low statistical power in missing person and DVI cases was published [591].

The most challenging kinship cases involve efforts to separate pairs of individuals who are typically thought to be genetically indistinguishable (i.e., monozygotic twins) or distant relatives (e.g., fourth cousins) where there is an increased uncertainty in the possible relationship. In some situations, somatic mutations may permit distinguishing monozygotic twins following whole genome sequencing – and this approach was successful in four of six cases reported recently [19]. The probative value of NGS data for distinguishing monozygotic twins was explored [592]. A unique case of heteropaternal twinning was reported where opposite-sex twins apparently had different fathers [593]. An impressive effort in kinship analysis using direct-to-consumer genetic genealogy information from 56 living descendants of multiple genealogical lineages helped resolve a contested paternity case from over a century and a half ago to identify the biological father of Josephine Lyon [594].

Techniques for identification of human remains continue to improve particularly with the capabilities of NGS and hybridization capture [595] and ancient DNA extraction protocols [596,597]. Studies have reported variation in skeletal DNA preservation [598] and retrospectively considered success rates with compromised human remains [599].

A simulated airplane crash enabled six forensic laboratories in Switzerland to gain valuable DVI experience with kinship cases of varying complexity [600]. The ISFG Spanish-Portuguese Speaking Working Group likewise conducted a DVI collaborative exercise with a simulated airplane crash to explore fragment re-associations, victim identification through kinship analysis, coping with related victims, handling mutations or insufficient number of family references, working in a Bayesian framework, and the correct use of DVI software [601]. Other groups have explored the capability of a particular software tool [602] or implemented rapid DNA analysis to accelerate victim identification [603]. The International Commission on Missing Persons (ICMP) has gained considerable experience with DNA extraction and STR amplification from degraded skeletal remains and kinship matching procedures in large databases [604]. To supplement the INTERPOL DVI Guide,[47] some lessons learned and experienced-based recommendations for DVI operations have recently been provided [605].

### 3.6. Non-human DNA testing and wildlife forensics

Non-human biological evidence may inform criminal investigations when animals or plants are victims or perpetrators of crime or the presence of specific material, such as cat or dog hair, may contribute to reconstructing events at a crime scene. Non-human DNA testing includes wildlife forensics and domestic animal species as well as forensic botany and has many commonalities and some important differences compared to human DNA testing [606–610]. Pollen analysis can assist criminal investigations [611,612]. The potential for and the barriers associated with the wider application of forensic botany in civil proceedings and criminal cases have been examined [613,614].

Mammalian species identification can assist in determining the origins of non-human biological material found at crime scenes through narrowing the range of possibilities [615]. New sequencing methods have been developed to assist species identification [616]. A multiplex PCR assay was developed to simultaneously identify 22 mammalian species (alpaca, Asiatic black bear, Bactrian camel, brown rat, cat, cow, common raccoon, dog, European rabbit, goat, horse, house mouse, human, Japanese badger, Japanese wild boar, masked palm civet, pig, raccoon dog, red fox, sheep, Siberian weasel, and sika deer) and four poultry species (chicken, domestic turkey, Japanese quail, and mallard) [617]. A number of other species identification assays have also been reported [618–620].

An important effort for harmonizing canine DNA analysis is an ISFG working group known as the Canine DNA Profiling Group, or CaDNAP.[48] The CaDNAP group published an analysis of 13 STR markers in 1184 dogs from Germany, Austria, and Switzerland [621]. Six traits for predicting visible characteristics in dogs, namely coat color, coat pattern, coat structure, body size, ear shape, and tail length, were explored with 15 SNPs and six InDel markers [622]. Canine breed classification and skeletal phenotype prediction has been explored using various genetic markers [623]. A novel assay using a feline leukemia virus was developed to demonstrate that a contested bobcat was not a domestic cat hybrid [624] and a core panel of 101 SNP markers was selected for domestic cat parentage verification and identification [625].

DNA tests have been developed to assist with illegal trafficking investigations involving elephant ivory seizures [626], falcons [627], and precious coral material [628]. Accuracy in animal forensic genetic testing was explored with interlaboratory assessments performed in

[47] See https://www.interpol.int/en/How-we-work/Forensics/Disaster-Victim-Identification-DVI.

[48] See https://www.isfg.org/Working+Groups/CaDNAP.

2016 and 2018 [629]. A collaborative exercise conducted in 2020 and 2021 by the ISFG Italian Speaking Working Group examined performance across 21 laboratories with a 13-locus STR marker test for *Cannabis sativa* [630]. A molecular approach was explored to distinguish drug-type versus fiber-type hemp varieties [631].

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsisyn.2022.100311.

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