# **Forensic Science**

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Review Contents	
Forensic DNA Analysis	4365
Collection, Characterization, Preservation, Extraction, and Quantitation of Biological Material	4366
Short Tandem Repeats	4367
Single Nucleotide Polymorphisms	4367
Y-Chromosome and X-Chromosome Analysis	4368
Mitochondrial DNA Typing	4369
Nonhuman DNA Typing Systems and Microbial Forensics	4369
DNA Databases, Mass Screens, and Mass Disasters	4369
Interpretation and Statistical Weight of DNA Typing Results	4370
General Reviews	4370
Trace Evidence	4370
Petroleum Products and Explosives	4370
Hairs, Fibers, Glass, and Paint	4371
Gunshot Residue Analysis	4372
Fingerprints	4372
Miscellaneous Trace Evidence	4372
Drugs and Poisons	4373
Ethanol and Volatiles	4373
Cannabinoids	4373
Morphine and Related Narcotics	4373
Amphetamines	4374
Benzodiazepines	4375
γ-Hydroxybutyrate and Related Compounds	4375
Miscellaneous Drugs	4375
Other Techniques	4376
Literature Cited	4377

This applications review aims to present a concise survey of articles appearing in publications that primarily appeal to forensic practitioners. With this objective, we have focused our attention on the following journals: Journal of Forensic Sciences, Forensic Science International, International Journal of Legal Medicine, Legal Medicine, Forensic Science Review, Forensic Science Communications, Analytical Chemistry, Electrophoresis, Science & Justice, Journal of the Canadian Society of Forensic Science, Journal of Analytical Toxicology, and BioTechniques as well as Chemical Abstracts Selects: Forensic Chemistry. Two new forensic journals have recently been launched as well: *Forensic Science, Medicine, and Pathology* (Humana Press) and *Forensic Science International: Genetics* (Elsevier). Our literature survey encompasses the period from January 2005 through December 2006. The format selected for this survey divides coverage into three distinct areas: forensic DNA analysis, trace evidence, and drugs and poisons. Within the scope of each of the areas, key articles have been selected to describe current forensic science practices in analytical chemistry and to outline relevant forensic science research interests. In accordance with the policy of the managing editor, we have strived to keep this review limited to important articles and to keep our discussions concise and meaningful.

### **FORENSIC DNA ANALYSIS**

The literature for forensic DNA analysis has expanded rapidly in the past few years as various technologies and genetic markers have been adopted, validated, and examined in numerous populations around the world. During 2005 and 2006, more than 600 papers and a number of books were published regarding DNA markers that are applied to human identity testing. Unfortunately, due to space constraints, only a selection of these articles will be highlighted below. It is worth noting that conference proceedings are available on-line for important meetings in this field including the International Symposium on Human Identification (http:// www.promega.com/geneticidproc/) and the International Society of Forensic Genetics (ISFG) (http://www.ics-publishing.com/ periodicals/ics) meetings. Volume 11 of Progress in Forensic Genetics, which contains the proceedings of the ISFG meeting held in September 2005, includes 285 brief articles covering current research on all aspects of forensic DNA typing (1).

New methods for detecting, preserving, extracting, and quantifying DNA are continually being developed and are aiding recovery of DNA from biological material found at crime scenes. Laser capture microdissection can be used to selectively recover sperm cells from sexual assault evidence (2). Whole genome amplification techniques are being explored as a potential way to enrich the amount of starting material in DNA tests (3). Realtime PCR has been introduced as an important tool to aid DNA quantitation especially with samples containing low amounts of DNA template (4). Short tandem repeat (STR) typing of autosomal markers with fluorescence-based detection is now almost universally used in forensic DNA laboratories worldwide (5). A large portion of the literature involves reporting STR allele frequencies from various populations. However, limited space in this review prevents a summary or description of the more than 250 population studies performed in 2005 and 2006.

Single nucleotide polymorphisms (SNPs) continue to be explored as potential supplements to STR markers already in use (6). SNPs are often thought to be useful in estimating ethnic origins of samples because their low mutation rate makes them more likely to become fixed in a particular allele configuration in a population compared to the more rapidly evolving STR markers. Due to a smaller target size (i.e., a single nucleotide change versus an accordion-like array of dozens of nucleotides with STRs), small PCR products can be created with SNPs, which translates into improved recovery of information from highly degraded DNA samples that have been fractured into small pieces. Miniature STR (miniSTR) loci are also being characterized for improved recovery of information from badly damaged DNA templates (7).

Information on uniparental lineage markers from the Ychromosome and mitochondrial DNA continues to accumulate in the literature. These lineage markers are also widely used for human evolutionary studies and the emerging field of genetic genealogy. The availability of commercial kits for Y-STR amplification has enabled more widespread usage of these important malespecific markers in forensic DNA laboratories. A number of X-chromosome STRs are also being investigated, and a web site has been established to compile information on X-STRs (8).

Nonhuman DNA plays a useful role in many forensic investigations. Tests have been developed for plant and animal DNA testing to associate victims or suspects to crime scenes. Detection and characterization of potential microbial agents used in biowarfare scenarios has given rise to the field of microbial forensics (9), which will continue to grow in importance with the threat of terrorism.

National DNA databases now collectively house millions of STR profiles around the world. With the demonstrated success of linking previous offenders to unsolved crimes they have committed, new legislation is expanding the number of samples that will be going into DNA databases of the future. In December 2006, the United States exceeded 4 million convicted offender profiles in the National DNA Index System of the FBI Laboratory's Combined DNA Index System (CODIS). Likewise, the United Kingdom has almost 4 million STR profiles in their national DNA database, which represents a significant portion of their active criminal population. The expansion of DNA databases has raised concerns over impact on citizens' civil liberties and individuals' genetic privacy, and a special issue on this topic was published in the *Journal of Law, Medicine, and Ethics* during the summer of 2006 (10).

Automation of laboratory techniques and data interpretation with expert systems has become increasingly important with the large numbers of DNA samples that need to be examined. Forensic DNA testing also aids investigation of missing persons cases and identification of mass disaster victims albeit with some extra issues unique to kinship and parentage analysis. At the end of the forensic DNA analysis section, we also list relevant papers on interpretation and statistical weight of DNA typing results published in 2005 and 2006 along with several general reviews of forensic DNA typing.

Collection, Characterization, Preservation, Extraction, and Quantitation of Biological Material. To aid simple collection of DNA from reference samples, a buccal collector was developed along with an elution procedure that can recover sufficient DNA for over 20 separate amplification reactions (11). A saliva sample collection method was described that yielded about 11.4  $\mu$ g/mL saliva although large amounts of bacterial DNA are present (12). A study of 42 buccal cell saliva samples stored for 7 years at room temperature, -20 °C, and -80 °C found that 76% of the samples failed when Identifiler STR kit amplification was attempted, suggesting that buccal cell DNA quantity and quality are severely compromised after lengthy storage on treated cards (13). Trehalose was reported to be a helpful preserving agent for highly dilute DNA samples from human placenta and gorilla fecal material (14).

Methods for body fluid identification using real-time PCR and messenger RNA profiling have been described (15,16). In addition, efforts have been made to estimate a bloodstain's age and see if the stain originated from a newborn (17). A review of automation issues was published discussing the roles of automated fluid handling robots, laboratory information management systems, and bioinformatics tools for data analysis (18). An automated PCR setup was demonstrated using a normalization wizard written for the Biomek 2000 robot to adjust DNA extract concentrations based on DNA quantitation values obtained with the AluQuant Human DNA Quantitation System (19). Improving efficiency of a small forensic DNA laboratory was discussed with robotic liquid handling and microchip capillary array electrophoresis systems (20). The Qiagen BioRobot EZ1 (21-23) and BioRobot M48 (23,24) were shown to work well for extracting DNA from standard forensic casework samples in an automated fashion. The QIAshredder/QIAamp DNA extraction kits were also evaluated (25). Methods were reported for obtaining trace DNA from bedding (26) and recovering nuclear DNA from hair shafts (27, 28), urine stains (29), and fecal material (30). Enzymatic digestion of cotton swabs improved recovery of DNA collected (31). The effects of heat and chemical treatment of bones on recovery of both nuclear and mtDNA were examined (32). Skeletal preparation techniques for recovering DNA from bone were explored along with different cleaning methods (33). The susceptibility of degraded human hair shafts to contamination by external blood, saliva, skin cells, and purified DNA was investigated (34). Microchip-based cell lysis and DNA extraction methods were developed for potential application to forensic casework (35). A simple extraction method for dried blood spots was described using incubation in 20 mM NaOH followed by a single wash in 10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0 buffer (36). The use of bleach to eliminate contaminating DNA on the surface of bones and teeth was explored (37). Procedures have been described for removing low amounts of contaminating DNA in "sterile" plasticware and water using ultraviolet irradiation (38). Extraction protocols have been optimized to aid recovery of DNA from low copy number DNA samples (39).

Laser capture microdissection is an important new way of selectively recovering specific cells, and when coupled with probes developed to label all cells containing a Y-chromosome, male cells can be isolated from mixtures with vaginal cells and used to recover a full STR profile when more than 20 cells are utilized (40). Several forensic case applications of laser capture microdissection were described (41). Fetal cells from an archival formalinfixed, paraffin-embedded abortion material were recovered in order to determine the obligate paternal alleles that demonstrated brother-sister incest (42). Different staining and extraction methods were evaluated to obtain efficient separation of spermatozoa from epithelial cells (2).

Whole genome amplification (WGA), prior to amplification of the specific regions of interest in forensic DNA analysis, was evaluated by a number of groups. Four different WGA methods were evaluated for locus and allelic dropout when typing the 13 CODIS STR loci (3). Routine production of autosomal and Y-STR profiles from one to two diploid cells was claimed with a modified improved primer extension preamplification PCR WGA method (43). An evaluation of multiple displacement amplification and improved primer extension preamplification found that these WGA methods performed poorly on degraded DNA and other forensically relevant samples (44). The effects of electron beam irradiation, which could possibly influence biological samples sent through the mail and exposed to pathogen-elimination irradiation measures, were explored (45). Multiple displacement amplification (MDA) was used to amplify single sperm cells (46), and STR typing success improved MDA from low-level DNA samples when molecular crowding techniques were used (47). A new set of DNA polymerases were described that have the potential to recover information from damaged DNA templates (48).

The NIST 2004 DNA Quantitation Study compiled accuracy and precision data for 19 different DNA quantitation methods across 80 different laboratories (49). Developmental validation studies were reported for the Quantifiler qPCR DNA quantitation assay marketed by Applied Biosystems (50). New quantitative PCR (qPCR) assays were described for detection of degraded DNA (51), simultaneous detection of nuclear and mtDNA targets (4), and total human and male DNA (52, 53). A popular Alu qPCR assay, previously performed with intercalating dyes, was converted to a probe-based assay (54). An alternative DNA quantitation technique involving the amplification of the sex-typing locus amelogenin was described (55). The levels of nuclear and mtDNA were measured in a number of different forensic evidence materials including plucked and shed head hairs (56). Examination of the impact of tannic acid levels on qPCR was performed to help analysts identify problematic samples possessing PCR inhibitors (57).

**Short Tandem Repeats.** Using genomic information from the Human Genome Project, the chromosomal locations of 18 commonly used autosomal STR loci and 11 core Y-STR loci were described along with a review of reported variant alleles, mutation rates, and web resources (5). Additionally, issues such as potential linkage of loci to disease genes, probabilistic predictions of sample ethnicity, and use of additional loci beyond the current core set were addressed in this STR review article. A computer program that graphically simulates the process of DNA extraction, PCR amplification, and visualization of STR alleles after electrophoresis was developed and used to generate random DNA profiles from allele frequency databases and proposed as a helpful tool to speed

future method validation (58). From 32 671 individuals examined with the 13 core U.S. STR loci, a total of 85 variants were identified at 12 of the 13 loci and characterized through DNA sequencing (59). Reliable typing of five Y-STR loci was demonstrated with locus-specific brackets composed of artificially created alleles smaller and larger than the common alleles seen for the tested loci (60). An examination of different PCR thermal cycling parameters found that a dramatic reduction in the amount of stutter product generated with several nonforensic STR loci could be obtained by lowering the denaturation temperature during PCR to 85 °C (61). A study of simulated power failure at various cycle numbers during PCR found that full and accurate STR profiles could be obtained if samples were subjected to additional PCR cycles following the power outage (62). An examination of 108 DNA samples and 52 negative controls with low-volume (1  $\mu$ L) PCR amplification reactions on chemically structured glass slides demonstrated that full STR profiles could be obtained from as little as 32 pg of template DNA (63). PowerPlex 16 and Profiler Plus kit STR typing results from 48 single-source samples, 16 mixture samples, and 17 nonprobative DNA samples were run on a 96channel microfabricated capillary array electrophoresis device and compared to those obtained on an ABI 310 Genetic Analyzer (64). Four different cancer cell lines were examined by STR typing through multiple generations of propagation to look at long-term stability of tissue culture cell lines (65). The informativeness of the 13 CODIS STR loci compared to 39 SNPs for reflecting an individual's ancestry was explored (66). Attempts were also made to reconstruct recent human phylogenies with five STRs (67).

Six new miniSTR loci, D1S1677, D2S441, D4S2364, D10S1248, D14S1434, and D22S1045, were characterized and allele frequency information provided for 474 individuals from U.S. population groups (68). Leaders of EDNAP (European DNA Profiling Group) and ENFSI (European Network of Forensic Science Institutes) recommended that miniSTRs be adopted as the way forward to increase both the robustness and sensitivity of DNA analysis and proposed as new core STR loci D10S1248, D14S1434, and D22S1045 (69). D14S1434, which has a low discriminating power, was later substituted with D2S441 (70). Success rates with a miniSTR assay utilizing D3S1358, FGA, TH01, VWA, and amelogenin showed an improvement in information recovery from 100 weak and/or degraded crime stains compared to commercially available STR kits (71). The forensic usefulness for a miniplex assay consisting of amelogenin, TH01, D2S1338, D18S51, D16S539, and FGA was demonstrated on 35 casework samples that showed loss of loci due to DNA degradation when previously analyzed by the commercial kit SGM Plus (72). Analysis of 31 bone samples showed improved success with miniplexes involving 12 of the 13 core STR loci when compared to amplification with the PowerPlex 16 STR kit (73). Through biotin labeling a subset of primers, 11 reduced size STR loci could be coamplified and then separated into nonoverlapping sets for two different runs on an electrophoretic separation system (74).

**Single-Nucleotide Polymorphisms.** There continues to be interest in exploring the usefulness of SNPs to aid various aspects of forensic investigations and human identity testing. A review of SNP typing methodologies was undertaken with nice illustrations of hybridization, primer extension, ligation, and cleavage allelic discrimination assays (75). A microarray system was described

for simultaneously detecting 21 mtDNA and 12 nuclear DNA SNP markers (76). A set of 24 highly informative SNPs covering each of the 22 autosomes and both sex chromosomes were reported with allele frequencies determined from 30 unrelated Korean individuals (77). A preliminary panel of 19 SNPs, selected from 195 markers, provided an average match probability of  $< 10^{-7}$  in most of the 40 populations examined (6). Validation experiments for a 21-locus SNP multiplex were reported along with an estimated discrimination power of 1 in 4.5 million using a UK Caucasian population database (78). A multiplex assay capable of simultaneously amplifying 52 autosomal SNPs was described along with allele frequencies from 700 individuals coming from 9 different populations (79). This 52plex assay was also demonstrated to work well on partially degraded DNA from crime casework samples due to having amplicons with a maximum size of 115 bp. An interlaboratory study organized by EDNAP compared analysis of artificially degraded blood and saliva samples with standard STR typing kits, a 21plex SNP assay, and two miniSTR assays and demonstrated that miniSTR systems were the most effective (80). The pros and cons of SNPs in forensic kinship investigations versus the currently used STRs were explored (81). The expected performance of SNPs in full trio and motherless paternity testing was evaluated, and it was noted that 70-80 SNPs would be required for deficient paternity cases to provide results similar to those that could be obtained with 16 STR loci (82). DNA methylation markers, however, may be able to help determine the parental origin of alleles in motherless cases, and an imprinted SNP locus rs220028 was examined as a model system for the use of epigenetic markers in forensic genetics (83). In addition, monozygotic twins, which share identical STR or SNP genotypes, have been shown to possess different methylation patterns as they get older (84).

Allele frequency differences in 43 Y-SNP markers were examined using 627 samples from males within the United Kingdom in order to explore the possibility of predicting the biogeographical origin of a man's ancestral paternity lineage (*85*). Four multiplex single base extension assays were developed to type 30 Y-SNPs in 292 samples from different regions of northwest Spain (*86*). Six PCR multiplexes were used to evaluate 37 Y-SNPs in 97 males from Europe, Asia, Africa, and South America and were also shown to work well on low molecular weight DNA fragmented by sonication (*87*). An EDNAP collaborative study involving 11 Y-SNPs compiled results for 535 samples across 6 different European populations and found that all 8 submitting laboratories obtained consistent and correct results on 4 blood samples (*88*).

**Y-Chromosome and X-Chromosome Analysis.** A number of on-line Y-STR databases are available as part of company web sites that provide haplotypes for the loci present in their specific Y-STR kits. The largest and most comprehensive Y-STR database for the minimal haplotype loci is the Y-Chromosome Haplotype Reference Database (YHRD; http://www.yhrd.org), which now contains over 46 000 haplotypes. The locations and sizes of these Y-STR databases have been summarized at http:// www.cstl.nist.gov/biotech/strbase/y\_strs.htm. The DNA Commission of the International Society of Forensic Genetics has issued an update of recommendations on the use of Y-STRs that addresses specific issues with nomenclature, locus selection for

forensic applications, mutations, and Y-STR haplotype frequency estimation (89). An overall mutation rate of  $\sim 0.002\%$  was reported across 17 Y-STR loci based on 27 029 allele transfers from 3026 father/son pairs (90). Mutation rates were also estimated for common Y-STR loci through evolutionary genetic dating that confirmed previously published father/son data (91). From a UK population study, 7 variant, 4 null, and 10 duplicated alleles were characterized in 13 different Y-STR loci (92). A single-source sample from Brazil was found to possess double peaks for DYS437. DYS439, and DYS389II (93). More Y-chromosomal duplications were reported along with suggestions on how to distinguish true male-male mixtures from locus duplications (94). The developmental validation for the 17plex Yfiler kit was described (95) and an N+3 stutter product characterized for the trinucleotide repeat locus DYS392 (96). A study involving 200 Minnesota Hispanics and Asian males found full concordance between typing results with the two most commonly used Y-STR systems: PowerPlex Y and Yfiler (97). Allele frequencies were reported for DYS441, DYS442, DYS443, DYS444, and DYS445 in 340 Japanese males and compared to some Chinese, U.S. Caucasian, and African American data sets (98). New multiplex assays for evaluating 43 additional Y-STR loci were reported and subjected to extensive validation studies (99). Gene diversities for 27 Y-STR loci were compared across all or a subset of 263 U.S. Caucasian, 260 African American, and 140 Hispanic samples (100). An analysis of 2517 individuals from 38 populations with 61 Y-SNPs was performed to look for paternal admixture among African-, European-, Hispanic, Asian-, and Native-Americans (101). It was noted that genealogical DNA testing involving Y-STRs can provide an inadvertent diagnosis of male infertility through detection of Y-chromosomal deletions (102). An analysis of 150 British surnames found that rare names were more likely to share Y-STR haplotypes, and a recommendation was made of combining Y-DNA profiles with existing DNA databases to narrow pools of suspects (103). In 24 of 26 child sexual assaults evaluated, Y-STR worked while autosomal STRs failed to give signs of victim-assailant DNA mixtures (104).

A new web site describing information on forensic X-chromosome research (http://www.chrx-str.org) was launched in 2005 (8). Haplotyping results from the three X-STRs DXS6801, DXS6809, and DXS6789 in 806 German males found 207 different haplotypes rather than the 1144 theoretically possible haplotypes based on single locus allele frequencies, suggesting that these loci are in strong linkage disequilibrium (105). Likewise, the loci DXS10079, DXS10074, and DXS10075, which are located within a 280-kb region, were studied in 781 German men and found to exhibit only 172 haplotypes when 2548 haplotypes were theoretically possible (106). An X-STR pentaplex with the loci DXS101, HPRTB, DXS8377, DXS981, and DXS6789 was developed and used to examine Chinese, Japanese, Thai, German, and Philippine population samples (107). A heptaplex X-STR assay was developed for DXS6789, HUMARA, DXS10011, DXS7423, HPRTB, DXS6807, and DXS101, and 268 females and 288 males were examined by five forensic laboratories in Italy (108). Two miniX-STR multiplexes were developed for the loci DXS7423, DXS6789, DXS101, GATA31E08, DXS8378, DXS7133, DXS7424, and GATA165B12 and used to demonstrate improved success rates with degraded DNA samples compared to conventional STR testing methods (109).

In a letter to the editor of the *International Journal of Legal Medicine*, three leading German X-STR scientists called for discontinuation of forensic DNA testing with the X-STR locus HumARA because of direct linkage to spinal and bulbar muscular dystrophy (*110*).

Mitochondrial DNA Typing. Concerns with the FBI mtDNA database sequence quality and the impact that it might have on accurately estimating frequency estimates for random matches were addressed (111). A practical guide was published for mtDNA sequence error detection and prevention (112). Systematic artifacts were described that give rise to phantom mutations during fluorescent mtDNA sequencing (113). Causes of errors in a proficiency testing mtDNA exercise were assigned to deficient electropherograms, errors during sequence editing, contamination, failure to detect heteroplasmy, and nomenclature problems (114). Another collaborative study examined a mixed stain of saliva and semen and found different relative amounts of nuclear and mtDNA in the saliva and semen (115). Denaturing highperformance liquid chromatography (HPLC) was proposed as a possible approach to separating mtDNA amplicon mixtures (116). A novel quantitation system for evaluation of mtDNA mixtures was developed with pyrosequencing (117). A 5-year retrospective review of mtDNA results on 691 hairs from forensic casework found that a full or partial mtDNA profile was obtained on >92% of the examined hairs (118). A simplified method for mtDNA extraction was described involving alkaline digestion to dissolve a hair in approximately 6 h (119). As hair samples degrade postmortem, levels of amplifiable mtDNA were found to decrease (120). A Linear Array Mitochondrial DNA HVI/HVII Region-Sequence Typing Kit was released by Roche Applied Science (Indianapolis, IN) and evaluated in 666 U.S. population samples (121) and 90 forensic casework samples (122). Electrospray ionization mass spectrometry has been used to determine base composition of mtDNA restriction fragments (123), and a 23plex PCR assay was developed to target 627 nucleotides in the coding region of the mtDNA genome (124). In response to criticism of limiting the evaluation of coding region mtDNA SNP sites to synonymous substitutions (125), effective strategies for forensic mtDNA analysis were explored and it was noted that 69% of variation in mtDNA protein coding genes from 2064 mtGenomes was found in the synonymous positions (126). Multiplex mtSNP assays were developed to help separate major European haplogroups (127) or subtype haplogroup H using 16 different SNPs (128) or 45 different SNPs (129) from the mtDNA coding region. A TaqMan assay for the highly discriminatory mtDNA control region SNP at position 16519 was used to evaluate mixture ratios in heteroplasmic samples (130). From a total of 208 individual hair samples evaluated from 26 mother-child pairs, 11 were found with point heteroplasmy (131). Length heteroplasmy was profiled in blood cells from 57 Korean donors (132). In a mtDNA control region CA dinucleotide repeat study, 4 alleles were observed in 500 Koreans with 3 samples possessing length heteroplasmy (133). The complete mtDNA control region was sequenced in 593 Koreans, which exhibited 494 haplotypes defined by 285 variable sites (134). Analysis was performed on the 1148 African American samples contained in the Scientific Working Group on DNA Analysis Methods (SWGDAM) forensic mtDNA data set (135) and 686 Hispanics also found in the SWGDAM mtDNA data set (136). A duplex primer set for amplifying about HVI and HVII control regions was evaluated along with different sequencing chemistries (137).

Nonhuman DNA Typing Systems and Microbial Forensics. Due to the fact that animal hair is frequently found at crime scenes, dog and cat DNA can aid forensic investigations. Several cases with canine STR and mtDNA analysis were described along with some of the population data underpinning the statistical analysis (138). Recommendations for animal DNA forensic testing, including basic analytical practices, data evaluation, and methodology validation, were developed based largely on experience gained with human identity testing over the past two decades (139). From a set of 49 candidate loci, 11 cat STRs were selected, developed into a single multiplex amplification assay, and characterized across 1043 cats representing 38 different breeds (140). Two PCR multiplexes that amplify 17 canine STRs were reported and used to develop a population DNA database containing 558 dogs from 16 different breeds and several mixed breeds (141). The probability of identity for a different set of 15 canine STRs was estimated at  $8.5 \times 10^{-8}$  based on examining 131 randomly selected dogs from the Innsbruck, Austria area (142). Allele sequencing was performed for the six canine STRs PEZ3, PEZ6, PEZ8, PEZ10, FHC2161, and FHC2328 in order to standardize their allele nomenclature and produce allelic ladders (143). Dog mtDNA control region sequences from 109 dog samples were generated and compared with 758 mtDNA sequences from previous studies (144).

Forensic botany and the use of plant DNA evidence was discussed in the context of aiding forensic death investigations (145). A method for testing *Cannabis sativa* suspect seeds was used involving chloroplast and nuclear DNA detection (146). The feasibility of DNA profiling different forensic soil samples was assessed (147). The challenges associated with microbial forensics were reviewed including various threats and accompanying challenges (148).

**DNA Databases, Mass Screens, and Mass Disasters.** The latest developments in forensic DNA policy are available at http://www.dna.gov, http://www.dnaresource.com, http://www.dnapolicy.net/, and http://www.aslme.org/dna\_04/ index.php. The possibility was explored of finding criminals through using DNA information of their relatives, a technique sometimes referred to as familial searching (*149*).

A case involving sexual murder was solved through a mass dragnet screening of 2335 men in Germany using a combination of STR and mtDNA information (*150*). A genomic matching technique involving sequence blocks from the major histocompatibility complex has been proposed to complement STR typing in high-volume crimes and DNA dragnets (*151*).

Challenges and issues behind identification of mass disaster victims were examined by several groups (*152,153*). Experiences from the 9/11/01 World Trade Center DNA victim identification efforts (*154*), skeletal remains identification from mass graves (*155*), and the 2004 tsunami (*156*) were described. On the fifth anniversary of September 11, 2001, the National Institute of Justice released a document entitled "Lessons Learned from 9/11: DNA Identification in Mass Fatality Incidents" (see http://massfatality.dna.gov/).

Interpretation and Statistical Weight of DNA Typing Results. The DNA Commission of the International Society of Forensic Genetics issued recommendations on the interpretation of mixtures (157). Software approaches to mixture interpretation have also been described such as PENDULUM (158) and leastsquares deconvolution (159). Approaches to interpreting low copy number DNA data involving mixtures and correcting for population substructure have been incorporated into a software program called LoComatioN (160). Theoretical considerations for interpretation of biological mixtures involving Y-STR haplotype data have been reported (161, 162). Using computer-generated conceptual mixtures, it was noted that based solely on the number of observed alleles, approximately 3% of three-person mixtures would possess a maximum of four alleles across all 13 STRs examined and could be mischaracterized as two-person mixtures (163). A new software tool for assessing quality of electrophoretic data from multiplex STR assays named Multiplex\_QA was described (164) and is freely available for download from http:// www.cstl.nist.gov/biotech/strbase/software.htm.

When obtaining a DNA profile from crime scene evidence, most laboratories simply report the match probability for an unrelated individual from relevant populations, yet the suggestion has been made to routinely report match probabilities for relatives (165). Theoretical calculations were made addressing the potential that one of a suspect's relatives matches his profile (166). The effect of linkage on DNA match probability calculations for siblings and half-siblings was also delineated (167). The reliability of the subpopulation model commonly used in match probability estimates was explored (168) as was the 2p rule often used to interpret partial DNA profiles when allele dropout may have occurred (169). A method has been suggested for permitting use of loci exhibiting allele dropout when kinship analysis is being performed (170).

A discussion of statistical analysis theory for paternity and kinship testing was provided with a worked example (171). Five examples of complicated deficient parentage cases were used to illustrate the value of additional STR loci in some situations (172). It was noted that approximately 25 STR loci appear necessary to achieve 95% confidence of detecting at least one genetic inconsistency indicative of nonparentage (173). A new method was suggested for assessment of the efficiency of genetic markers used in paternity studies (174). Paternity index calculations were described that incorporated co-ancestry coefficients for both autosomal and X-chromosomal results (175). Some possible pitfalls were described when motherless paternity analysis was performed (176). The ability to determine half-sibling relationships when maternal genotypes are known was explored (177). A summary of nonpaternity rates published in the literature found data ranging from 0.68 to 30% although the general rate is likely in the 1-5% range (178).

**General Reviews.** A bibliometric analysis of 14 210 forensic science and legal medicine publications between 1981 and 2003 was performed (*179*). The technology behind forensic DNA typing was described (*180*). Legal perceptions of forensic DNA profiling were also reviewed (*181*).

#### TRACE EVIDENCE

The term "trace evidence" refers to materials expected to transfer to a crime scene or to a suspect in very small quantities. Fiber, paint, and glass evidence examination is included in this category as is the examination of debris collected from fires and explosions to detect residues of ignitable liquids, explosives, or both. Also covered in this section is the analysis of gun shot residue (GSR), techniques for the development of fingerprints, and other miscellaneous analyses considered trace analyses. During 2005 and 2006, more than 250 papers and a number of books were published regarding trace evidence analyses, only a selection of which is presented below.

Petroleum Products and Explosives. The ASTM E-30 Committee on the Forensic Sciences published five consensus documents on the analysis of ignitable liquid residues (182-186). The recovery of fingerprint marks from soot-covered glass fire debris was reported (187). Covariance mapping was used in the analysis of ignitable liquids by gas chromatography/mass spectrometry (GC/MS) (188). A review of the literature on the analysis of vegetable oil residues from fire debris samples highlighting the spontaneous ignition of vegetable oils was reported (189). Adsorption saturation effects on chromatographic distortion was studied when activated charcoal passive headspace sampling was used in fire debris analysis (190). "Electronic noses" and a dynamic headspace sampler were evaluated as tools in field sampling during fire investigations (191). A study describing the distribution and range of products and gases released following the pyrolysis of biomass residues samples was reported (192). Nanosecond time-resolved spectroscopy was used to study the fluorescence imaging of petroleum-based products (193). The influence of the type of ignitable liquid, type of burned material, time of burning, and availability of air on the possibility of detection of ignitable liquid residues was reported (194). Adsorption saturation and chromatographic distortion effects from the use of passive headspace sampling using activated charcoal in fire debris analysis was studied (195) as was the presence of waterproofing compounds considered ignitable liquids over some time after the deposition of the waterproofing materials (196). The identification of Isopar H in vinyl flooring was also reported (197).

A book devoted to information processing from detectors used in neutron radiation analysis of explosives was published (198). The training and testing of explosive detection canines for the detection of triacetone triperoxide was reported (199). The analysis of explosives using terahertz time-domain and Raman spectroscopy was reported (200). Standoff detection of explosive materials such as 2,4,6-trinitrotoluene (TNT) by differential reflection spectroscopy was reported (201). A micromachined chemiluminescent system for explosives detection was described (202). A sorbent-coated micropreconcentrator was studied for the enhanced trace detection of explosives and chemical agents (203) and explosives detection was reported using fast neutron transmission spectroscopy (204). Hierarchically structured nanocomposite films were found to act as highly sensitive chemosensory materials for TNT detection (205). Remote detection of explosives was accomplished using pulsed laser surface fragmentation followed by mid-infrared laser spectroscopy detection (206) and by using desorption electrospray ionization (DESI) mass spec-

trometry about 3 m from the mass spectrometer (207). Laserinduced breakdown spectroscopy (LIBS) in combination with a conventional mine prodder was applied for remote detection of explosives and mine casing materials (208), and the detection of explosive materials such as TNT was achieved by differential reflection spectroscopy (209). The detection of explosives using heated microcantilever sensors was reported (210) as was the detection of 2,4-DNT using deep ultraviolet Raman spectroscopy equipped with a 244-nm laser excitation source (211). The detection of the chemical signatures from TNT and 2,4-DNT buried in sand at various ambient conditions was studied (212). A standoff IR detection system was designed from commercially available IR equipment (213), and the detection of explosives and explosives-related compounds was reported by using by single photon laser ionization time-of-flight mass spectrometry (214). A review of the analysis of explosives using microchip electrophoresis and conventional capillary electrophoresis was published (215). The determination of peroxide-based explosives such as triacetone triperoxide (TATP) and hexamethylene triperoxide diamine was reported using liquid chromatography with on-line infrared detection (216). Other methods for the determination of peroxide-based explosives were also reported (217). The application of ion mobility spectrometry (IMS) in cases of forensic interest for the detection of analytes such as environmental pollutants, warfare agents, explosives, herbicides, pesticides, and petroleum products as well as for the detection of prescription and illicit drugs was described (218). Solid-phase microextraction (SPME) was coupled to an IMS detector for the first time to detect the explosives TNT and their signature compounds including the taggants 2-nitrotoluene, 4-nitrotoluene, and 2,3-dimethyl-2,3-dinitrobutane (219, 220). SPME was also used to identify the dominant odor chemicals emanating from explosives for use in developing optimal training aid combinations and mimics for canine detection (221) and to differentiate between different smokeless powders using SPME-GC/MS and SPME-GC-ECD in order to improve training aids for explosives detection canines (222). SPME coupled to GC was also used in the analysis of explosives in soil (223). Chemical ionization tandem mass spectrometry (CI-MS/MS) was used to improve on the detection of organic explosives (224). Multiphase extraction sampling of explosives in unsaturated soils was studied (225), and low-limit photoacoustic detection of solid 1,3,5-trinitro-1,3,5-triazocyclohexane (RDX) and TNT explosives was achieved using a carbon dioxide laser excitation source (226). Characterization of high explosive particles using cluster secondary ion mass spectrometry was reported (227). A report on the composition profile of low explosives from cases encountered in India was published (228). Micellar electrokinetic chromatography (MEKC) and capillary electrochromatography analyses of 14 nitroaromatic explosives and their degradation products in seawater was reported (229). The application of thin-layer chromatography (TLC) for low-level analysis (LOD ~70 ng) of trinitrotoluene residues in environmental samples was also reported (230). An evaluation of the bioavailability of the explosive metabolites hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) in soils using passive sampling devices was conducted (231). The determination and characterization of organic explosives using porous graphitic carbon and liquid chromatography-atmospheric pressure chemical ionization (APCI) mass spectrometry was reported (232). Surface detection of chemical warfare agent simulants and their degradation products was also reported (233). The application of neutron generators for high explosives, toxic agents, and fissile material detection was studied (234). The desorption electrospray ionization of explosives on surfaces was conducted to evaluate the sensitivity and selectivity enhancements by reactive desorption electrospray ionization (235). The characterization and identification of explosives and explosive residues was achieved using GC/ MS, an FT-IR microscope, and HPTLC (236). Photoassisted electrochemical detection was used to analyze environmental samples containing explosives (237). The extraction and analysis of trace amounts of cyclonite (RDX) and its nitroso metabolites in animal liver tissue using gas chromatography with electron capture detection (GC-ECD) was reported (238). LIBS was used as a chemical sensor for explosives (239). The examination of the organic explosives TNT, RDX, pentaerythritoltetranitrate (PETN), and tetryl (2,4,6-trinitrophenylmethylnitramine) were examined using IMS (240). Raman spectrometry of explosives with a no-moving-parts, fiber-coupled spectrometer was used to compare the effect of different excitation wavelengths on the detection of the explosives (241). Rapid on-site environmental sampling and analysis of propellant stabilizers and their decomposition products by portable sampling and thin-laver chromatography kits was reported (242). A platform for on-site environmental analysis of explosives using high-performance liquid chromatography with UV absorbance and photoassisted electrochemical detection was reported (243). The detection of TNT at parts-per-trillion level by using a newly prepared SPR immunosensor, anti-TNPh-KLH antibody (2,4,6-trinitrophenyl-keyhole limpet hemocyanine) based on indirect inhibition method was reported (244). Detection of explosives on solid surfaces by thermal desorption and ambient ion/molecule reactions was studied (245). Trace detection of explosives with low vapor emissions by laser surface photofragmentation-fragment detection spectroscopy with an improved ionization probe was also reported (246). Liquid chromatography electrospray tandem mass spectrometric and desorption electrospray ionization tandem mass spectrometric analysis was used to detect chemical warfare agents collected from common surfaces (247). The determination of elemental sulfur in explosives and explosive residues was achieved by using gas chromatography/mass spectrometry (248). A comparison of smokeless powders and mixtures was conducted using capillary zone electrophoresis (249). A field diagnostic test for the improvised explosive urea nitrate was reported (250). The differentiation between different composition C-4 samples was based on the analysis of the mineral oil added to the C-4 formulation (known as the "process oil" using high-temperature GC/MS (251). The discrimination of match heads was achieved by comparing the elemental profiles using inductively coupled plasma-atomic emission spectrometry (ICP-AES) (252).

Hairs, Fibers, Glass, and Paint. Microchip electrophoresis was used for the analysis of mitochondrial DNA in forensic and ancient DNA studies (253). Degradation of postmortem human hair was correlated to the utility of mitochondrial DNA analysis of that hair (254). Donor dependence on the success of generating STR genotyping of keratinized hair was reported (255). Three methods for the effective extraction of DNA from human hair were

studied (256). A summary of mitochondrial DNA analyses of 691 forensic hair cases was reported (257). The sorption and accumulation of the explosives TNT, RDX, PETN, TATP, and EGDN in hair during exposure to their vapors was studied (258). The role of stable isotopes in human identification in order to determine the variability of isotopic signals in human hair and nails was conducted (259). Hair analysis—a tool in biomedical, environmental and forensic sciences: a review of literature published after 1989 (260) and Hair and human identification, a review (261) were published. The Scientific Working Group on Materials (SWGMAT) published guidelines for the forensic examination of hair (262) and for training a forensic fiber examiner (263).

The damage caused to textile fibers by the action of two types of heat was reported (264). The determination aluminum, beryllium, and cationic surfactants were analyzed using viscose and cotton cloth test strips encapsulated into a polymeric film (265). Raman spectroscopy was used in the forensic analysis of black/ gray and blue cotton fibers (266). Analysis of fiber dyes by liquid chromatography-mass spectrometry (LC-MS) with electrospray ionization was studied to discriminate between dyes with indistinguishable UV-visible absorption spectra (267). The utility of thin-layer chromatography and UV microspectrophotometry in the analysis of reactive dyes released from wool and cotton fibers was reported (268). The crystallinity of acrylic fibers was evaluated by using a glass refractive index measurement system (269). The effect of exposure to the elements on the forensic characterization by infrared spectroscopy of poly(ethylene terephthalate) fibers was reported (270).

A review on the advances in the forensic analysis of glass fragments with a focus on refractive index measurements and elemental analysis was published (271). The SWGMAT guidelines for the examination of glass including an introduction to forensic glass examinations, the collection, handling, and identification of glass, the initial examinations of glass, the examination of glass fractures, glass density determinations, glass refractive index determinations, and the elemental analysis of glass were also published (272). A number of different papers on the elemental analysis of glass by different methods were published including a guide for the quantitative elemental analysis of glass using laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) (273), an evaluation of a standard method for the quantitative elemental analysis of float glass samples by LA-ICPMS (274), and the validation of a method for the analysis of float glass using LA-ICPMS (275). Sampling strategies for the analysis of glass fragments by LA-ICPMS were also reported (276, 277). The determination of iron in glass by laser ablation and solution sampling using a dynamic reaction cell-ICPMS was also reported (278). Analysis of glass fragments by laser ablation-inductively coupled plasma-mass spectrometry and principal component analysis was reported (279). The application of LIBS for the discrimination of glass samples of forensic interest was reported for the first time (280) and compared to LA-ICPMS results. A second report on the application of LIBS to glass analysis was also published (281). The application of total reflection X-ray fluorescence (TXRF) spectrometry to the analysis of small glass fragments was reported (282). WDXRF, EPMA, and SEM/EDX was used to study the quantitative chemical analyses of small glass samples (283). The discrimination of small glass fragments was demonstrated using the nondestructive technique of high-energy synchrotron radiation X-ray fluorescence spectrometry (SR-XRF) (284). A study of the performance and utility of annealing in forensic glass analysis was reported (285). The forensic discrimination of sheet glass samples combining refractive index measurements and elemental analysis with a SR-XRF were also reported (286).

Raman spectroscopy was used in the analysis of paint evidence case studies (287) and for the analysis of automotive paint samples (288). An evaluation of pyrolysis-gas chromatography-mass spectrometry for paint samples analyses was reported (289). Infrared chemical imaging of multilayered paint chips was also reported (290).

Gunshot Residue Analysis. A summary of the Federal Bureau of Investigation Laboratory's GSR symposium was published (291). Ion exchange was used to separate Sb from cloth for subsequent analysis of GSR using neutron activation analysis (292). The application of X-ray fluorescence analysis was used in the investigation of the composition of gunshot residues (293). Fast mapping of gunshot residues was accomplished by batch injection analysis with anodic stripping voltammetry of lead at the hanging mercury drop electrode (294). A study was conducted to determine the potential for being able to identify primer gunshot residue within the particulate residues produced during the firing of a revolver using black powder propellant and a percussion cap primer. (295). Lead isotope analysis was accomplished using multiple-collector inductively coupled plasma mass spectrometry to examine the potential variability of lead isotope compounds present in shooting incident investigations (296). Portable, generator-based XRF instruments were evaluated for the nondestructive analysis of GSR and other potential evidence at crime scenes (297). A report on the ENFSI proficiency testing program for the identification of GSR by SEM/EDX was published (298).

Fingerprints. A report on the fingerprint evidence in the Madrid train bombing case was published (299). The identification of oxidation products of squalene in solution and in latent fingerprints was conducted by using electrospray ionization (ESI)-MS and LC/APCI-MS (300). The detection of visible and latent fingerprints by micro-X-ray fluorescence was reported (301). The detection and mapping of latent fingerprints by LIBS was reported (302). Poly(cyanoacrylate) formations were studied by Raman spectroscopy analysis in order to investigate the enhancement of imaging latent fingerprints (303). Nanosecond resolution imaging of fluorescent samples from fingerprint was studied (304). Fluorescence optimization and lifetime studies of fingerprints treated with magnetic powders was reported (305). The detection and enhancement of latent fingerprints was reported using visible absorption and luminescence chemical imaging (306) and infrared chemical imaging (307). Changes in the lipid composition of latent fingerprint residues with time after deposition on a surface was studied (308). A novel fingerprint reagent, Genipin, was studied for its colorimetric and fluorogenic activity (309).

**Miscellaneous Trace Evidence.** Several reports on the chemical characterization and persistence of human scent were published (310-312). Drop size and impact velocity determinations from circular bloodstains were conducted (313). The age of an individual at death was reported to be determined from

radiocarbon present as a result of nuclear bomb tests (314). A method was reported for the simultaneous and direct determination of the trace elements Al, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Sr, Ba, La, and Pb in ancient tooth samples and its distribution in dentin and enamel by slurry sampling fluorination-assisted electrothermal vaporization inductively coupled plasma mass spectrometry (315). The spatial distribution of lead in human primary teeth as a biomarker of pre- and neonatal lead exposure was reported (316). Trace element determinations in human cortical and trabecular bones were reported (317). Nonaqueous capillary electrophoresis with red light emitting diode absorbance detection for the analysis of basic dyes was reported (318). Ink identification by time-offlight secondary ion mass spectroscopy was reported (319). Analysis of Indian blue ballpoint pen inks tagged with rare-earth thenovltrifluoroacetonates was conducted using inductively coupled plasma-mass spectrometry and neuron activation analysis (320). Forensic classification of ballpoint pen inks using HPLC and infrared spectroscopy (IR) with principal components analysis and linear discriminant analysis was reported (321). The characterization of ballpoint pen inks by thermal desorption and gas chromatography-mass spectrometry was reported (322). The classification and dating of black gel pen ink by ion-pairing HPLC was reported (323). Photofading of ballpoint dyes on paper was studied by laser desorption/ionization (LDI) and MALDI MS (324). Visible and near-infrared chemical imaging methods were used for the analysis of selected forensic samples (325). The use of LDI mass spectrometry in the analysis of inks in questioned documents was also reported (326). The use of Raman spectroscopy for the analysis of blue gel pen inks was reported (327). Heterogeneity and aging of ballpoint pen inks inside of pen cartridges was studied (328). The forensic significance of bullet lead compositions was reported (329). The forensic analysis of soils and sediments taken from the cast of a footprint was also reported (330). Forensic analysis of soil and sediment traces was also studied using scanning electron microscopy and energydispersive X-ray analysis (331). The extraction of capsaicin and other compounds from aerosol defense sprays was also reported (332). A report on the current state of forensic chemistry education and research including the areas of trace evidence analysis was also published (333).

### **DRUGS AND POISONS**

Ethanol and Volatiles. Chloroform was measured in biological fluids by headspace GC-FID using 1,1,1-trichloroethane as the internal standard (334). An unusual postmortem urine/blood alcohol concentration ratio was determined in a victim (335). A proposed decision rule provides a sound statistical basis for establishing fitness-for-purpose in duplicate breath alcohol measurement (336). Blood samples were analyzed for ethanol by headspace (HS) gas chromatography (337). Ethanol and acetaldehyde were determined using HS-GC-FID with capillary columns (338). Quantification of ethanol in blood was carried out by HS-GC-FID (339). Postmortem ethanol concentrations were determined in pericardial fluid and bone marrow aspirate by HS-GC/ MS (340). Aromatic solvents detected in the blood of a drugfacilitated sexual assault victim were detected using GC-FID and GC/MS in full scan mode after liquid-liquid extraction (341). The precision of breath alcohol testing in the field using the Intoxilyzer 5000C and the effect of truncation has been studied (342). An article discusses the use of urine as a biological specimen for determination of alcohol in clinical and forensic toxicology and discusses factors that might influence variability in the urine/blood concentration ratio of alcohol (343). Pulsed electrochemical detection following reversed-phase (RP) LC was applied recently to the detection of ethyl glucuronide in the urine (344). Ethanol and 1-propanol were quantified in postmortem blood (345). Interpreting results of ethanol analysis in postmortem specimens has been reviewed (346). A discussion on determining the air/water partition coefficient to employ when calibrating forensic breath alcohol test instruments has been published (347). Ethanol concentrations were measured in right cardiac blood, left cardiac blood, and peripheral blood by HS GC-FID (348).

Cannabinoids. A procedure for the determination of tetrahydrocannabinol (THC)-COOH in hair using 2-D gas chromatography (GC  $\times$  GC) coupled to mass spectrometry (GC-GC/MS) is described for the first time (349). A headspace solid-phase microextraction combined with a GC/MS method was developed for the extraction and analysis of cannabinoids from Cannabis samples (350). The stable carbon and nitrogen isotopic ratios were measured in marijuana samples (351). A HPLC procedure for the determination of  $\Delta^9$ -THC in human plasma has been described (352). Quantification of  $\Delta^9$ -THC-COOH was performed after alkaline hydrolysis, liquid./liquid extraction, derivatization, and analysis by GC/MS using single ion monitoring. (353). SPE followed by HPLC was used for the determination of  $\Delta^9$ -THC in human plasma (354). Oral fluid was tested for the presence of THC using the OraLine IV s.a.t. device and GC/MS (355). The Correlation of  $\Delta^9$ -tetrahydrocannabinol concentrations determined by LC-MS/MS in oral fluid and plasma from impaired drivers has been studied as well as the evaluation of the on-site Draeger DrugTest (356). Plasma was analyzed for THC and 11-nor-9carboxy-∆-tetrahydrocannbinol GC/MS (357). THC and cannabidiol were determined in human urine using SPME and LC-MS using APCI (358). The determination of 11-nor- $\Delta^9$ -tetrahydrocannabinol-9-carboxylic acid in oral fluid specimens is described using an oral fluid collection device and GC/MS (359-361). Chromatographic and spectroscopic data was determined for 16 different major cannabinoids from C. sativa plant material as well as 2 human metabolites of  $\Delta^9$ -tetrahydrocannabinol using UV absorbance, IR-spectral analysis, GC/MS, and fluorescence spectrophotometric analysis (362). Plasma THC and THC-COOH concentrations were measured by GC/MS (363).

Morphine and Related Narcotics. LC-ESI-MS/MS was used to determine morphine and metabolites in urine (364). A RP-HPLC method has been developed and validated for the determination of methadone and metabolite EDDP in biological fluids (365). EMIT and GC/NPD was used to detect heroin metabolites in blood and saliva (366). HPLC with UV detection was developed for the determination of morphine in urine of heroin abusers (367). A GC/MS method for the determination of methadone, heroin, cocaine, and their metabolites in urine using selected ion monitoring (SIM) was developed (368). A novel monolith microextraction method coupled with capillary zone electrophoresis (CZE) was proposed for rapidly determining a mixture of opiates in human urine (369). A LC-ESI-MS/MS has been presented for quantitative determination of opioids and their metabolites in blood plasma after an automatically performed solidphase extraction (SPE) (370). A research-based bioanalytical/

forensic science laboratory experiment has been designed that makes use of a commercial available 125I-labeled RIA kit (Coat-a-Count) is described (371). After consumption of poppy seeds, various substances were detected in urine or blood samples using an immunoassay and LC-MS/MS (372). HPLC-diode array detection (DAD) and SPE was used for the simultaneous determination of morphine and other opiates in plasma (373). Sweat patches were analyzed for opiates by SPE and GC/MS (374). ELISA has been used for the detection of methadone in postmortem specimens (375). GC/MS and HPLC were used to analyze heroin synthesis products (376). Opiates and cocaine and its related metabolites were analyzed in pericardial fluid by GC/MS (377). Capillary electrophoresis (CE) was used to analyze heroin in urine specimens (378). The determination of morphine in the larvae of Calliphora stygia using flow injection analysis and HPLC with chemiluminescence detection has been reported (379). A fast LC/MS method has been developed for the simultaneous determination of methadone, its metabolite, and alprazolam, in human plasma (380). Hydromorphone was detected and quantitated in biological specimens using GC/MS (381). Opiates were screened by immunoassay and GC/MS (382). Morphine metabolites in urine were detected by HPLC-MS/MS (383). A method was developed that used HPLC-PDA detection and mass spectrometric detection to separate diacetylmorphine- and caffeinerelated compounds (384). A HPLC method was developed for the determination of morphine in plasma (385). Oxycodone was determined by immunoassay and GC/MS (386). A method to determine morphine in human liver and kidney using GC/MS has been reported (387). The performance of an enzyme immunoassay for the detection of oxycodone and its primary metabolite, oxymorphone, in urine was completed by comparing the results to GC/MS (388). A comparison of ELISA and GC/MS detection of methadone and metabolites in human hair has been reported (389).

Amphetamines. Amphetamines were detected in oral fluid using Microplate EIA and confirmed by GC/MS (390). Amphetamine, methamphetamine, ephedrine, pseudoephedrine, phentermine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethylamphetamine (MDEA), and ketamine were identified in blood using UPLC/MS (391). The suitability of segmental hair analysis of MDMA to monitor past chronic exposure to the drug was investigated (392). MDMA and metabolites were detected in an infant's urine (393). The synthetic origin of methamphetamine samples have been determined by <sup>2</sup>H NMR spectroscopy (394). The profiling of impurities in methamphetamine using headspace solid-phase microextraction and GC/MS has been described (395). A rapid and selective HPLC method using monolithic columns was developed for the separation and quantification of amphetamines in ecstasy tablets (396). MDA, MDMA, and metabolites were determined in oral fluid using SPE and GC/ MS-EI with SIM (397). Determination of phenylalkylamine derivatives has been performed using a Twister device for solid-phase microextraction (398). Pulsed splitless injection has been used for impurity profiling of methamphetamine crystals by GC or GC/ MS (399). A rapid and selective HPLC method using monolithic columns was developed for the separation and quantification of the principal amphetamines in ecstasy tablets (400). A study was designed to characterize the mass spectrometric data resulting

from sequential derivatizations of commonly abused amphetamines (401). The applicability of CE with a UV detector using highly sulfated  $\gamma$ -cyclodextrin as a chiral selector for analysis of impurities in seized methamphetamine has been studied (402). Impurities in methamphetamine were investigated by GC/MS and GC-FID (403). MDMA in sample tablets demonstrated various colors and logos. The appearances and contents of ecstasy tablets were analyzed and the amount of MDMA was quantified by GC/ MS (404). MDMA, methamphetamine, and related drugs were determined in hair by GC/MS using selective ion monitoring after derivatization with trifluoroacetic anhydride (405). Amphetamines were determined in serum by flash trifluoroacetylation with MBTFA and GC/MS (406). Class identity assignment for amphetamines using neural networks and GC-FT-IR data has been reported (407). A sensitive semi-microcolumn HPLC with a fluorescence detection method was developed for the determination of MDMA and related compounds in human hair (408). UPLC/MS/MS was used to separate and identify amphetaminetype substances, common and novel designer analogues (MDA, MDMA, PMA, 4-MTA, MBDB), and ketamine (409). Analysis of amphetamine, methamphetamine, and MDMA by micellar capillary electrophoresis using cation-selective exhaustive injection has been reported (410). A sensitive GC/MS method was developed and validated for the simultaneous measurement of MDEA, MDMA, and its metabolites in human urine (411). A rapid analysis of methamphetamine and its metabolites in urine was performed by GC/MS (412). Ecstasy tablet samples were extracted by diethyl ether under alkaline conditions and then analyzed by GC/MS (413). Artifacts in the GC/MS profiling of underivatized methamphetamine hydrochloride have been discussed (414). A microchip-based liquid-liquid extraction for the GC analysis of urine for amphetamine-type stimulants has been developed (415). A method using an immunoaffinity column and ESI-LC-MS was developed for the quantitation and enantiomeric determination of (S)-(+)-methamphetamine in urine (416). Amphetamine and related compounds were quantified in urine by GC/NPD or GC/ MS. (417). Methamphetamine was detected in urine samples when analyzed by GC/MS after SPE and derivatization (418). MDMA and related compounds were detected in hair, urine, and various postmortem specimens using extraction, derivatization, and GC/MS with deuterated analogues of the analytes as internal standards (419). Methamphetamine seizures were analyzed using GC/MS (420). A report describes the identification of a number of byproducts, which are produced during the Wacker oxidation of safrole to 3,4-methylenedioxyphenyl-2-propanone using  $\rho$ -benzoquinone and palladium chloride when methanol is utilized as the solvent (421). The study of the biotransformation of 2.5dimethoxy-4-bromamphetamine and identification of its metabolites in urine has been described using GC/MS with various ways of derivatization (422). A method for the simultaneous determination of methamphetamine and amphetamine in urine using a HPLC column-switching method has been reported (423). A CZE method for the simultaneous chiral determination of the enantiomers of N,N-dimethylamphetamine, methamphetamine, ephedrine, pseudoephedrine, and methylephedrine has been reported (424). Direct analysis of methamphetamine, amphetamine, and *p*-hydroxymethamphetamine in urine was achieved by cationselective exhaustive injection and sweeping micellar EKC (425). Chiral separation and quantification of (R/S)-amphetamine, (R/S)-

S)-methamphetamine, (R/S)-MDA, (R/S)-MDMA, and (R/S)-MDEA in whole blood has been accomplished by GC/EI-MS (426). A novel chiral derivatization agent, (2S,4R)-N-heptafluorobutyryl-4-heptafluorobutoyloxyprolyl chloride, was used for the indirect resolution of amphetamine enantiomers using GC/MS-NICI (427). A method was developed and validated using headspace GC-FID for the identification of 1-phenylpropene in urine (428). Acidic and enzymic hydrolysis with  $\beta$ -glucuronidase from Escherichia coli and Helix pomatia was evaluated for the detection of MDMA metabolites in urine (429). An on-line derivatization method utilizing GC with furan CI-MS/MS for screening of amphetamines in urine has been reported (430). The spectral absorption features of methamphetamine were studied by terahertz time-domain spectroscopy (431). Positive ion ESI-MS was used to detect MDMA derivatives in fomalin tissue (432). TLC was used to identify the active components in ecstasy tablets (433). Illicit ecstasy tablets were analyzed by GC/MS (434). LC-MS/MS was used to elucidate an isomer of MDMA (435). The use of turbulent flow chromatography for on-line sample cleanup of urine samples for detection of amphetamines was developed (436). A sensitive and specific CIMS method for the simultaneous detection and quantification of amphetamine, opiates, and cocaine and metabolites in human postmortem brain was developed (437). A CZE method was developed for the analysis of amphetamine analogs (438). Positive ion ESI-MS, ESI-MS/MS, and ESI-MS/ MS/MS were used to detect MDMA derivatives in fomalin tissue (439). A method for screening for and simultaneously quantifying 10 2,5-methylenedioxy derivatives of amphetamine and phenethylamine in human whole blood, using CE with DAD has been reported (440). A method using a SPE and ion-pair LC-ES-MS/ MS was developed for determination of amphetamine and methamphetamine in urine samples (441). An amperometric immunosensor for the specific and simple detection of MDA and its analogues, MDMA and MDEA, in saliva and urine was developed (442). A novel fiber coated with  $\beta$ -cyclodextrin derivative has been used for headspace solid-phase microextraction of ephedrine and methamphetamine in human urine (443). DESI-MS was used as a simple and rapid way to analyze drug tablets and powders without sample preparation (444). A rapid and simple method has been described for the analysis of the D- and L-isomers of seven methamphetamine-related compounds and the D-isomer of pseudoephedrine by CE/MS with direct injection of urine (445).

Benzodiazepines. Cases from drivers suspected of driving under the influence and suspects of violent crime were analyzed to show the relationship between behavior and blood flunitrazepam concentration (446). An electrochemical procedure using SPE and carbon-paste electrodes was developed to analyze benzodiazepines and metabolites in plasma and urine (447). HPLC was used to detect benzodiazepines and barbiturates in biological material (448). SPE and electrospray LC-MS/MS/MS was used to detect benzodiazepines in urine (449). Fast GC/NICI-MS combined with rapid and nonlaborious sample preparation has been presented for the simultaneous determination of benzodiazepines and  $\alpha$ -hydroxy metabolites, zaleplon and zopiclone, in whole blood (450). SPE followed by LC-MS/MS was used to detect benzodiazepines and metabolites in urine (451). The sensitivity and selectivity of an enzyme multiplied immunoassay technique (EMIT II Plus) relative to LC-MS/MS was determined for benzodiazepines detected in oral fluid and urine by LC-MS/

MS (452). ELISA and LC-MS/MS was used for the detection of benzodiazepines in hair (453). An anti-diazepam, molecularly imprinted polymer has been synthesized and used to extract diazepam and other benzodiazepines from hair samples via a molecularly imprinted solid-phase extraction protocol (454). A sweeping technique, in conjunction with micellar electrokinetic chromatography, for the simultaneous determination of flunitrazepam and its major metabolites has been described (455). A simple and fast procedure was developed for the simultaneous determination of benzodiazepines in whole blood using LC-APCI-MS (456). Extraction and analysis of flunitrazepam and metabolites in blood and urine has been done by LC-PDA and GC/MS using butyl SPE columns (457). A LC-MS/MS method has been developed and validated for the determination of benzodiazepines and/or their metabolites in human urine (458). A method involving HPLC with dual-electrode electrochemical detection in the redox mode has been developed for the determination of nitrazepam in serum (459).

γ-Hydroxybutyrate and Related Compounds. A procedure for the detection of exogenous GHB in blood by GC/combustionisotope ratio mass spectrometry (GC-C-IRMS) following liquidliquid and solid-phase extractions and derivatization of GHB to the di-TMS derivative before analysis has been published (460). A GC/MS/MS method for determining GHB in blood and urine has been developed (461). GHB was measured by GC/MS in urine (462). A GC/MS/MS method for determining trace concentrations of GHB in blood and urine has been developed (463). GHB levels were measured by GC/MS in urine (464). GHB was analyzed in biological fluids and stored samples by liquid-liquid extraction with adipic acid internal standard, MSTFA derivatization, and assayed on a GC/MS operating in EI SIM mode (465). IMS was investigated as a method of screening urine and breath for the presence of GHB and related drugs (466). Endogenous GHB in biological samples was analyzed using a HS-GC/MS method (467). GHB was determined in tissue and biological fluids as the trimethylsilyl derivative (GHB-di-TMS) and GC-PCIMS (468)

Miscellaneous Drugs. An analysis method for determination of tetramethylenedisulfotetramine (tetramine) in human urine by gas chromatography/flame thermionic detection coupled with a direct immersed solid-phase microextraction was developed (469). A sensitive, specific, and reproducible method for the quantitative determination of the anabolic metandienone in human hair has been developed using liquid-liquid extraction, derivatization, and GC/MS (470). A novel method based upon HPLC/ion trap mass spectrometry detection with electrospray ionization interface was developed for the identification and quantification of colchicine in plasma or whole blood (471). 2,4-Dinitrophenol was determined in biological fluid by HPLC and GC/MS (472). The detection of drugs in hair has been reviewed (473). Optical properties of illicit drugs and active pharmaceuticals have been compiled (474). Phloroglucinol has been recommended for the selective detection and determination of methomyl in biological materials by TLC (475). A formula has been proposed for estimating the postmortem interval based on the determination of hypoxantine in vitreous humor using HPLC (476). Methods for the detection of drugs in hair have been reviewed (477). Setraline and its degraded products were extracted from embalming fluid by liquid-liquid extraction using chloroform and then analyzed by GC/MS using

the electron impact ionization mode (478). The application of hair as a biological indicator of drug use/abuse or of chronic exposure to environmental toxicants has been reviewed (479). A CE method for direct bromide quantification in serum has been developed (480). An overview has been given of the existing standards and guidelines for analytical toxicology (481). Amlodipine was measured by liquid chromatography-atmospheric pressure photoionization-mass spectrometry (482). Tizanidine was determined in postmortem biological fluids and tissues (483). Quantitation of propoxur in blood and urine was carried out using SPE and HPLC with a photodiode array UV detector (484). LC-MS/MS and SPE was used for the analysis of  $\beta$ -agonists and  $\beta$ -antagonists in blood (485). The chromatographic techniques used in the detection of drugs of abuse in hair have been reviewed (486). A simple, rapid, and reliable method for direct bromide quantification in serum based on CE has been reported (487). A collaborative study was conducted to determine the prevalence of cannabinoids, opiates, cocaine metabolites, and amphetamines in blood samples from drivers killed in road accidents (488). Dextromethorphan and its metabolites were analyzed in urine using TLC and GC/MS (489). Quaternary ammonium drugs and herbicides in human whole blood were analyzed by LC/MS/MS with positive ESI following SPE (490).

A rapid and sensitive method was developed for the simultaneous identification and quantitation of ketamine and its major metabolite in hair using GC/MS (491). A new and sensitive method to determine ticlopidine in whole human blood using GC/ EI-MS and proadifen as the internal standard has been described (492). A simultaneous determination of 20 antidepressant drugs in human plasma was developed using LC/MS with a sonic spray ionization method (493). A review of the physiological basics of hair growth, mechanisms of substance incorporation, analysis methods, result interpretation, and practical applications of hair analysis for drugs and other organic substances has been published (494). A method to detect trimeprazine in hair was developed based on testing by LC-MS/MS (495). A sensitive ELISA was developed for the detection of fentanyl in serum and urine (496). The detection of colchicines in blood has been accomplished by using LC-MS/MS (497). Blocking agents, such as succinylcholine, pancuronium, and tubocurarine, have been detected in a forensic setting using LC-MS/MS on a tandem quadrupole/time-of-flight instrument (498). An LC-MS screening method was developed to detect atractyloside (499). 5-Methoxy-N,N-diisopropyltryptamine and its two metabolites, 5-hydroxy-N,Ndiisopropyltryptamine and 5-methoxy-N-isopropyltryptamine were identified in blood and urine by LC-MS (500). The rapid and sensitive identification and determination of urine by ESI-MS after reduction of chromate has been reported (501). A new method has been described for the qualitative and quantitative analysis of cyanide in human whole blood, which involves the conversion of cyanide into hydrogen cyanide and its subsequent HS-SPME and detection by GC/MS in SIM mode (502). A simple, rapid, reliable, and validated analytical method suited for forensic examination of diesel fuel No. 2 in biological specimens by GC-FID and GC/MS has been described (503). Fentanyl was quantitated in blood (504). Duloxetine was detected in postmortem specimens by a basic liquid-liquid extraction and GC-NPD and GC/MS (505). Atropine and scopolamine were detected in hair by LC-MS/MS (506). Psychiatric drugs were detected in

liver, gastric contents, and blood collected from heart and femoral sites using LC-MS (507). Analysis of hallucinogenic constituents in Amanita mushrooms by GC/MS has been reported (508). Strychnine was determined in urine by using a liquid-liquid extraction followed by LC-MS/MS /APCI (509). Carboxyhemoglobin was measured using visible spectrophotometry, and the detection and quantitation of gasoline was performed by means of GC-FID and confirmation was performed using GC/MS (510). A procedure for the determination of parathion in human whole blood and urine using direct immersion SPME and GC/MS has been presented (511). A LC-ESI-MS/MS method for the determination of colchicine in autopsy samples has been described (512). The detection of phencyclidine (PCP) in nails has been reported (513). A method that involves GC/MS based on twostep derivatization was used to determine ketamine and norketamine in human hair (514). An ELISA has been validated for the detection of buprenorphine in urine samples (515). A method was developed for screening human biological samples for poisonous anions using CE employing indirect UV detection (516). An automated procedure based on isotope-dilution GC/MS for the accurate and rapid determination of CN in whole blood has been reported (517). A GC/MS procedure was applied for the analysis of  $\alpha$ - and  $\beta$ -thujone with cyclodecanone as internal standard (518). The extraction and analysis of ropinirole from whole blood using solid-phase cartridges has been presented (519). A GC/MS method for the simultaneous determination of N,N-diethyl-mtoluamide (DEET) and permethrin with <sup>2</sup>H<sub>10</sub>-phenanthrene as an internal standard and a separation external standard HPLC method for pyridostigmine bromide determination in human plasma were developed (520). a-Methyltryptamine was determined in biological fluids by SPE and GC/MS (521). A LC-MS screening method for the detection of sedative-hypnotics in serum is described (522). Quantitation of atenolol, metoprolol, and propranolol in postmortem human fluid and tissue specimens was accomplished by LC/ APCI-MS (523). The thin-layer chromatographic determination of diazepam, phenobarbitone, and saccharin in toddy samples has been reported (524). A simple and modern method for the simultaneous analysis of nitrite and nitrate in whole blood was devised by ion chromatography with an autosuppressor and a conductivity detector (525).

Other Techniques. GC/MS has been used to detect drugs of abuse in blood samples (526). Isotopic ratio mass spectrometry was used to differentiate the origin of drugs of abuse (527). Immunochemical findings in urine have been checked with GC/ MS confirmation methods (528). A rapid LC-MS/MS method for drug confirmation has been described using reversed-phase gradient elution chromatography with identification of drugs based on their multiple reaction monitoring transitions, retention time, and coelution of stable isotopic analogues where available (529). An ESI-MS/MS library of 800 compounds has been developed (530). Ion mobility spectrometry has been used for the analysis of drugs, sweat samples, and explosives (531). Trace elements in hair have been determined by synchrotron X-ray fluorescence analysis (532). Urine was digested with urease, and the drugs were analyzed by GC/MS in the scan mode after SPE and acetylation (533). Accurate mass measurements were made by ESI using Fourier transform ion cyclotron resonance mass spectrometry to elucidate the structures of sildenafil, tadalafil, and vardenafil analogues that were found in products marketed as

dietary supplements (534). LC-ESI-MS/MS method was used for identification and quantification of polar metabolites of explosives (535). The combined use of ESI-QqTOF-MS and ESI-QqTOF-MS/MS with a mass spectral library search for the identification of therapeutic and illicit drugs has been evaluated (536). Comparison with HPLC-DAD and GC/MS for the analysis of drugs in urine and blood samples showed that linear ion trap tandem mass spectrometry could identify most of the compounds (537). A rapid urinalysis system based on SPE-LC-MS/MS with an in-house postanalysis data management system has been developed for the simultaneous identification and semiguantitation of opiates, methadone, amphetamines, 1,4-benzodiazepines and their metabolites, and ketamine (538). Cation-selective exhaustive injection and sweeping micellar electrokinetic chromatography was directly used to test drugs in human urine (539). Drug recovery from a new oral fluid collection device was been assessed (540). A desorption system was evaluated for use in combination with GC/MS for determining the presence of basic drugs in forensic samples (541). An evaluation of the dynamics of external contamination of hair with cocaine while developing performancetesting materials for Federal Drug-Free Workplace Programs was reported (542). Samples of Napoleon's hair have been tested by ICPMS (543). CE with a  $\text{Ru}(\text{bpy})_3^{2+}$  electrochemiluminescence (ECL) detection system was established for the determination of contamination of banknotes with controlled drugs, and a highefficiency on-column field-amplified sample stacking technique was also optimized to increase the ECL intensity. (544). A method was developed for drug screening in urine by LC-ESI-TOFMS (545). A rapid, simple, and highly efficient on-line preconcentration method using MEKC for the analysis of abused drugs (546). A review has been given on forensic applications of the thermal desorption MS/MS (547). Surface-activated chemical ionization was employed for the analysis of drugs by ion trap mass spectrometry (548). The use of ion trap mobility spectrometry for direct analysis of illicit drugs at trace level in sweat was studied (549). An evaluation of two immunoassav systems with GC/MS has been performed for drug screening (550). Applications of CE in forensic science have been reviewed (551). HPLC separation of drugs at elevated pressure has been described (552).

Drugs of abuse were analyzed by LC-MS, after solid-phase extraction in the presence of the deuterated analogues (553). This paper describes a novel method that applies on-line, backextraction, field-amplified sample injection to the extractants by solvent microextraction in CZE (554). The application of IRMS in forensic science has been reviewed (555). Urine immunoassay results and the results of hair tests by means of GC/MS were compared (556). An evaluation of an oral fluid drug testing system as an on-site screening tool for vitreous humor samples collected during postmortem examinations was performed using GC/MS for confirmation (557). HPLC methods to directly detect drug glucuronides in biological matrixes have been reviewed (558). Qualitative and quantitative analysis of illicit drug mixtures on paper currency using Raman microspectroscopy has been reported (559). A method for determining the purity of reference drug standards and the routine analysis of illicit drugs and adulterants using <sup>1</sup>H NMR spectroscopy has been presented (560).

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