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Forensic Science

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Review Contents

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*. Our survey encompasses the period from January 2003 through December 2004. Because of the normal delays in the abstraction of journal articles by *Chemical Abstracts*, some work covering this period will inadvertently be omitted. Hopefully these references will be included in the next biennial review. The format selected for this survey divides coverage into three distinct areas: forensic DNA analysis, trace evidence, and drug 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 and validated. During 2003 and 2004, close to 1000 papers were published regarding DNA markers that are applied to human identity testing. A selection of these articles will be highlighted below. In addition, a number of books on the subject have been published recently (*1*−*10*). Conference proceedings are also available on-line for the International Symposium on Human Identification (http://www.promega.com/geneticidproc/) and the International Society of Forensic Genetics meetings (http://www.ics-publishing.com/periodicals/ics) (see also refs *4* and *5*).

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. Real-time PCR has been introduced as an important tool to aid DNA quantitation especially with samples containing low amounts of DNA template (*11*).

Short tandem repeat (STR) typing of autosomal markers with fluorescence-based detection is now almost universally used in forensic DNA laboratories worldwide. The ABI 310 and ABI 3100 genetic analyzers, which are the primary instrument platforms for STR typing, have been reviewed along with issues surrounding sample preparation, detection, and interpretation of STR profiles (*12*). Commercial STR kits enable routine multiplex amplification of as many as 15 STR markers in a single assay (*13*, *14*). Protocols

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for analyzing DNA samples with commercial STR kits have been described (*15*). A large portion of the literature involves reporting STR allele frequencies from various populations. However, due to space limitations, these population studies will not be fully listed.

A new section has been created in this review for single-nucleotide polymorphisms (SNPs), which have been discussed by some in the field as potential additional markers to or replacements for commonly used STRs due to the capabilities of generating small PCR products that can better handle highly degraded DNA samples.

Information on uniparental lineage markers from the Y-chromosome 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. New commercial kits from Y-STRs have become available in the past two years enabling more widespread usage of these important male-specific markers. A number of X-chromosome STRs are also being investigated.

Nonhuman DNA continues to play a useful role in many forensic investigations. New tests have been developed for plant and animal DNA testing to associate victims or suspects with crime scenes. Detection and characterization of potential microbial agents used in biowarfare scenarios 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 2004, the United States exceeded two million convicted offender profiles in the National DNA Index System of the FBI Laboratory's Combined DNA Index System (CODIS). The United Kingdom has almost three million STR profiles in their national DNA database, which represents a significant portion of their active criminal population. The U.K. database continues to be the most effective in the world at linking repeat offenders to their crimes and aids more than 1000 criminal investigations each week (http://www.ojp.usdoj.gov/nij/pdf/uk_finaldraft.pdf). 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 tested. At the end of the forensic DNA analysis section, we also list relevant papers on interpretation and statistical weight of DNA typing results along with several general reviews of forensic DNA typing.

Collection, Characterization, Preservation, Extraction, and Quantitation of Biological Material. A simple buccal DNA collector was developed for direct collection of buccal cell samples (*16*). A study evaluating FTA and three other commercial papers as DNA storage media found little difference in their ability to obtain typeable STR results after storage for 19 months (*17*). Efforts have been made to use FTA cards for more widespread collection of crime scene evidence (*18*) and for collecting, archiving, and processing DNA from wildlife samples (*19*). Sperm cells from sexual assault evidence spread on microscope slides can be collected with laser-capture microdissection to perform reliable STR testing (*20*). A microfabricated device has been developed to separate sperm and epithelial cells using the differential physical properties of the two cell types (*21*). A molecular biology approach using messenger RNA profiling is being developed for sensitive and specific tests of various body fluids including saliva (*22*). The ABAcard HemaTrace test was found to have a limit of detection of 0.07 μg of hemoglobin/mL and shows specificity for human blood along with higher primate and ferret blood (*23*). In an examination of seminal fluid from 227 males with 3 different detection methods, the prostate-specific antigen detection method was found to be the most reliable (*24*).

A review of strategies to overcome inhibition and generate PCR-compatible samples was published (*25*). The addition of aluminum ammonium sulfate proved helpful to prevent the co-purification of inhibitors with DNA from soil samples (*26*). The affinity resin thiopropyl Sepharose 6B has been used to remove PCR inhibitors (*27*). Two studies were published examining the effects of electron beam irradiation on buccal cell DNA (*28*, *29*). Both studies concluded that while electron beam irradiation, at doses that might be used to irradiate letters or packages sent through the mail to kill possible pathogens, reduces the yields and quality of DNA extracted from buccal cell collections; the short tandem repeat DNA typing systems used in human identity testing (*28*, *29*) and mitochondrial DNA (*29*) could still be successfully amplified.

Laboratory personnel should be appropriately gowned during interactions with samples prior to PCR amplification (*30*). A "staff elimination database", containing the genotypes of laboratory personnel, is often searched prior to concluding that a generated DNA profile accurately reflects the evidence and is not due to contamination from laboratory personnel (*31*). The Forensic Science Service noted that sporadic contamination of consumables used in DNA testing, such as the small tubes in which the PCR amplification is performed, can introduce extraneous DNA profiles (*32*). Since PCR contamination can be tube-specific, one study that explored the impact of contamination on casework reporting guidelines noted that negative controls run with a batch of samples cannot provide complete confidence that the associated batch of extracted casework material is contaminant-free (*33*). DNA extractions and setting up PCR reactions should be performed in a dedicated laboratory similar to what is done in the "ancient DNA" field (*34*).

A simple closed tube DNA extraction method has been demonstrated with a thermal stable protease that looks very promising (*35*). The Qiagen solid-phase extraction approach can be performed with centrifugation or vacuum manifolds in single-tube (*36*) or 96-well plate formats (*37*). Higher-throughput approaches, though, are incorporating 96-well extraction formats with products such as the QIAamp 96 DNA Blood Kit (*38*). Promega's DNA IQ extraction method has been automated on the Beckman 2000 robot workstation and implemented into forensic casework by the Virginia Division of Forensic Science (*39*). It was noted that each robotic system has different capabilities and should be carefully assessed in order to meet the needs and goals of one's own laboratory environment (*40*).

A review of various DNA quantification methods was published (*11*). Promega Corporation has developed a human DNA quantitation system that enables sensitive detection of DNA (*41*). The AluQuant assay possesses a range of 0.1−50 ng for human DNA and can be automated on a robotic liquid handling workstation (*42*). End-point PCR tests using a single STR locus (*43*) or other

region of the human genome, such as an *Alu* repeat (*44*, *45*), have been developed. Quantifiler and Quantifiler Y are two commercial real-time PCR kits that became available from Applied Biosystems in November 2003. The amount of male DNA in a female−male mixture can be estimated using the Quantifiler Y kit, which targets a region of the SRY gene on the Y-chromosome. Other real-time quantitative assays have also been developed and implemented in forensic laboratories for quantifying DNA samples prior to PCR amplification including one that targets the flanking region of the STR locus TH01 (*46*) and ones that probe multicopy *Alu* sequences (*47*, *48*). Real-time PCR assays have also been developed to estimate mitochondrial DNA content in forensic and ancient DNA samples (*49*) and to perform rapid sex determination on forensic evidence (*50*). An interlaboratory test of 74 laboratories conducted by the National Institute of Standards and Technology (NIST) found a 10-fold range of reported concentrations when evaluating various DNA quantification methods (*51*). The performance of spectrophotometric DNA quantification, PicoGreen fluorometric analysis, and a novel real-time PCR method specific to the human BRCA1 locus were compared (*52*).

Quantification of DNA from various regions of human teeth found the root body yielded the greatest amount of DNA (*53*). An examination of skeletal and cardiac muscle with a PCR-based assay found approximately 3000 and 7000 copies, respectively, of mitochondrial DNA relative to nuclear DNA in these tissues (*54*).

Short Tandem Repeats. Literally thousands of polymorphic microsatellites have been characterized in human DNA and there may be more than a million microsatellite loci present depending on how they are counted (*55*). Computer searches of the recently available human genome reference sequence have attempted to comprehensively catalog the number and nature of STR markers in the genome (*56*, *57*). One of these studies found 23 676 tetranucleotide STRs in the human genome (*57*).

New commercial STR kits have become available in the past several years for both autosomal and Y-chromosome markers. AmpFlSTR Identifiler and Profiler Plus ID kits possess an extra unlabeled D8S1179 reverse primer to prevent allele dropout in Asian populations due to a mutation in the middle of the primer binding site (*58*). The availability of five dye detection systems has enabled development of multiplexes capable of amplifying and analyzing 20 or more STR loci (*59*, *60*). Primer concentrations were noted to be one of the largest factors in a multiplex PCR reaction determining the overall yield of each amplicon (*61*). Steps to designing STR multiplex assays were outlined (*61*, *62*).

The ABI 3700, ABI 3100, and MegaBACE 1000 capillary array electrophoresis systems were evaluated for STR typing (*63*). Precision of the ABI 3100 was noted when using the Identifiler kit (*64*). Microfabrication methods are now being applied to develop miniature, microchip-based laboratories, or so-called "Labs-on-a-Chip" (*65*). Research is ongoing to improve separation speeds and ease of use with the hope that soon microchip CE devices will be used routinely for rapid DNA analyses (*66*).

An interlaboratory study demonstrated that there is an inverse relationship between the size of the locus and successful PCR amplification from degraded DNA samples, such as those obtained from a crime scene or a mass disaster (*67*). The preparation of degraded human DNA used in this interlaboratory study was also described (*68*). Through redesign of PCR primers to be close to the STR repeat region, reduced size PCR products have been generated with common STR loci to yield higher recovery of information from degraded DNA templates (*69*). These so-called "miniSTRs" have been demonstrated to work more effectively than commercial STR kits with DNase I artificially degraded DNA (*70*). A concordance study performed on over 500 samples run with miniSTRs and a commercial kit found only a few discrepancies primarily with D13S317 and VWA (*71*). Reduced size STR assays have helped make possible some of the World Trade Center victim identifications from burned and damaged bone samples (*72*).

Two studies found that the Global Southern sizing algorithm worked well and maintained a better precision than Local Southern sizing in situations when temperature fluctuations can occur (*73*, *74*). The Forensic Science Service low-copy number (LCN) approach was confirmed with consensus profiles from separate single-cell PCR experiments matching the actual profile of the cell donor (*75*). Whole genome amplification prior to PCR was found to exhibit the same stochastic fluctuations with low levels of DNA including allele dropouts from STR loci at 50 and 5 pg of starting material (*76*). A graphical simulation model was developed to aid in predicting stochastic variation that occurs with LCN DNA testing (*77*). Different types of triallelic patterns were characterized (*78*). Stutter product amounts and heterozygote peak height balance were assessed on 468 database and 275 casework single-source samples (*79*).

The German DNA profiling group (GEDNAP) reported on its successful blind proficiency testing program (*80*, *81*). It was noted that a blind external proficiency test is generally considered the most effective at monitoring a laboratory's abilities but can be rather expensive and time-consuming to arrange and conduct (*82*, *83*). Reference DNA samples were reported to be crucial to the validation of any DNA testing procedure (*84*).

The use of expert systems involving computer software to automatically review DNA profiles is growing. In a comparison of alleles calls from 2048 STR profiles between manual review with Genotyper and automated review with TrueAllele, only one significant difference was observed when the analyst using Genotyper interpreted a spike as a DNA peak at D8S1179, but TrueAllele correctly designated it as a spike (*85*). Validation studies with 290 676 STR markers found CompareCalls^{SM} software, an automated allele concordance analysis system from Myriad Genetics, to be at least as accurate as 100% human technical review of STR profiles (*86*).

Allele frequencies for common alleles at 15 different STR loci across 700 samples from the 3 major U.S. population groups were published (*87*). Information on the 13 CODIS STR loci across several thousand U.S. Caucasian and African American samples along with almost 1000 North American Hispanics was reported (*88*).

STR markers have been used in a number of applications besides human identification. A study with 203 matched related donor−recipient pairs ranked 27 different STRs, including the 13 CODIS core loci, in terms of their ability to detect chimeric mixtures (*89*). Not surprisingly, the loci with the highest heterozygosities, namely, Penta E, SE33, D2S1338, and D18S51, worked the best. Normal and cancerous tissue from an individual can vary fairly dramatically in some instances in terms of their STR allele peak heights (*90*). Genome scans for disease gene

mapping are routinely performed with around 400 STRs covering the human genome at 5−10 centiMorgan (cM) distances (*91*). The Marshfield genome scan STR markers include five of the CODIS loci: D7S820, D8S1179, D13S317, D16S539, and TPOX. Studies with this same set of data have identified particular STR loci that are effective indicators of ancestral origin (*92*).

Single-Nucleotide Polymorphisms. Forensic DNA scientists often use additional markers as the need arises to obtain further information about a particular sample. The DNA working group of the European Network of Forensic Science Institutes (ENFSI) and the U.S.-based Scientific Working Group on DNA Analysis Methods (SWGDAM) jointly issued an assessment that "it is unlikely that SNPs will replace STRs as the preferred method of testing of forensic samples and database samples in the near to medium future" in national DNA databases (*93*). However, SNPs have the potential to be used in other ways to aid investigations, such as predicting a perpetrator's ancestral background (*94*) and improving resolution of common mitochondrial DNA haplotypes (*95*).

Several members of the European forensic DNA typing community launched a project in 2003 known as SNP*for*ID that is developing SNP assays to directly aid forensic DNA analysis (*96*). Most likely a multiplexed battery of 50−100 autosomal SNPs will be required to match the same powers of discrimination and mixture resolution capabilities now achieved with 10−16 STR loci (*93*). Progress is being made in the area of multiplex PCR amplification with a 35plex involving Y-chromosome SNPs (*97*) and a 39plex autosomal SNP assay (*98*). The most common detection method is allele-specific primer extension using the SNaPshot kit and multiccolor capillary electrophoresis detection. A 17plex Y-SNP assay has been demonstrated on MALDI-TOF mass spectrometry (*99*).

SNPs continue to get a lot of press in the media and scientific community due in large measure to the International HapMap project, which is a follow-up on the Human Genome Project and includes a plan to type 270 individuals from African, European, and Asian populations with approximately 1 million SNPs (ref *100*; see http://www.hapmap.org/). To aid in cataloging SNP loci of forensic interest in a common format, the National Institute of Standards and Technology has set up a forensic SNP web site: http://www.cstl.nist.gov/biotech/strbase/SNP.htm (*93*). SNP typing technologies have also been reviewed (*101*−*103*).

Y-STR Typing, Gender Identification, and X-Chromosome Analysis. A detailed analysis of the completed Y-chromosome reference sequence was described in the June 19, 2003 issue of *Nature* by researchers from the Whitehead Institute and Washington University (*104*). The Y-chromosome has become a popular tool for tracing historical human migration patterns through male lineages (*105*) and is growing in popularity for the emerging field of genetic genealogy (*106*). Approximately 250 biallelic markers have been characterized on the Y-chromosome (*107*, *108*). Additionally, more than 200 Y-STRs are now known (*108*) largely due to a fairly comprehensive screen of genomic sequence information (*109*). Using Y-chromosome-specific PCR primers can improve the chances of detecting low levels of the perpetrator's DNA in a high background of the female victim's DNA (*110*, *111*). Successful recovery of Y-STR profiles from six postvasectomized azoospermic semen samples has been demonstrated (*112*). A special issue of *Forensic Science Review* devoted to Y-chromosome analysis and its use in forensic science was published in July 2003.

Most Y-STR population studies and databases have examined the minimal haplotype loci of DYS19, DYS389I/II, DYS390, DYS391, DYS392, DYS393 and the multicopy locus DYS385 (*108*). The additional loci DY438 and DYS439 were added to the minimal haplotype loci as the recommended core set of loci for the U.S. by the Y-chromosome subcommittee of the FBI's SWGDAM (*113*, *114*). A number of commercial Y-STR kits were developed in the past two years to enable forensic practitioners to use these core loci in male-specific amplifications. These kits including ReliaGene's Y-PLEX 6 (*115*), Y-PLEX 5 (*116*), Y-PLEX 12 (*117*), PowerPlex Y from Promega (*118*), and Yfiler from Applied Biosystems. ReliaGene reported use of Y-STRs on 188 forensic samples from 2000 to 2003 with their Y-PLEX 6 and Y-PLEX 5 kits (*119*). Shewale et al. (*117*) found that male−female mixtures down to 1:400 still resulted in the male component amplifying at the Y-STR loci. Successful recovery of the male Y-STR profile in greater than 1000:1 female/male mixtures has also been demonstrated (*118*). Fetal microchimerism was demonstrated to not be an issue with Y-STR typing (*120*).

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). The information in this database comes from 89 collaborating institutions located in 36 different countries (*121*). The Asian database portion of the YHRD was described (*122*). A number of on-line Y-STR databases are also available as part of company web sites that provide haplotypes for the loci present in their specific Y-STR kits. These locations and sizes of these databases have been summarized at the following web site: http://www.cstl.nist.gov/biotech/strbase/y_strs.htm.

Several studies have shown that additional loci are helpful in resolving common Y-STR haplotypes obtained with the minimal haplotype loci. Addition of the multicopy marker DYS464 to the minimal haplotype loci increased the number of different haplotypes in a set of 135 Austrian males from 110 to 122 (*123*). In another study, 25 samples that possessed an indistinguishable most common minimal haplotype could be subdivided into 24 different groups (only one pair could not be resolved) with the addition of DYS438, DYS439, DYS464, DYS458, DYS460, and DYS437 (*124*). Statistical studies have also been performed to compare various combinations of Y-STR loci to the minimal haplotype in order to determine the best order in which to apply the markers (*125*).

Several studies have been conducted to examine mutation rates among the commonly used Y-STR loci. Most studies have focused on the minimal haplotype loci. Two different approaches have been used: deep-rooted pedigrees (*126*) and male germline transmissions from confirmed father/son pairs (*127*, *128*). Bonne-Tamir et al. (*126*) examined 74 male samples that spanned 139 generations. Allele gains were more common than allele losses as the mutations occur with not only locus-specific but also allele-specific differences in mutation rate (*127*). Triplicated DYS385 alleles have also been reported (*128*). It has been demonstrated that with a nested PCR approach, the "a" and "b" alleles for DYS385 can be amplified separately (*129*, *130*).

A number of Y-SNP typing technologies were reviewed (*108*). Allele-specific primer extension and allele-specific hybridization methods were compared and found to be in complete concordance on almost 4000 Y-SNP allele calls (*131*).

Analysis of Y-chromosome STRs and mitochondrial DNA has been used for genetic genealogy studies as well (*132*). An interesting study of more than 2100 males from Central Asia found that approximately 8% of those studied had a unique Y-chromosome lineage that they believe is from Genghis Khan (*133*).

The primary method for gender determination in human identity testing continues to be amplification of amelogenin from both the X and Y chromosome (see ref *1*) even though there is occasional erroneous gender identification with this assay (*134*). A mobile element-based assay for human gender determination using a Y-chromosome Alu insertion has been described (*135*).

X-Chromosome analysis can be valuable in parentage and kinship testing (*136*). The X-STR markers DXS10011, DXS101, DXS6789, DXS7132, DXS8377, and DXS9895 have been studied in several hundred individuals from Taiwan (*137*). DXS6800, DXS101, and DXS8377 were examined in Austrian and German males and females (*138*). The loci DXS6803 and DXS6793 were evaluated in a Chinese population (*139*). Sequence structure and population data for X-STRs DXS7423 and DXS8377 were clarified (*140*). GATA172D05, HPRTB, DXS8377, DXS101, and HumARA were analyzed in 150 males and 150 females from Korea (*141*).

Mitochondrial DNA Typing. A special issue of *Forensic Science Review* devoted to forensic applications of mitochondrial DNA (mtDNA) was published that included review articles on heteroplasmy (*142*), methods for detection of single-nucleotide polymorphisms (*143*), application of phylogenetics to mtDNA haplogroup definitions (*144*), and challenges facing degraded skeletal remains identification (*145*). The forensic frontiers of mtDNA were explored in additional reviews (*146*, *147*).

The SWGDAM mtDNA subcommittee published guidelines for mtDNA sequence analysis (*148*). The FBI Laboratory described their efforts in validating mtDNA sequencing on the ABI 3100 genetic analyzer and noted their 10:1 rule for contamination detection is conservative and reliable (*149*). A commercial kit for screening common polymorphisms in the mtDNA control region was released by Roche Analytical Systems based on a linear array solid-phase probe hybridization technology (*150*). A tabulation of success rates for obtaining reportable mtDNA sequencing results across different skeletal materials found that ribs and femurs work best (*145*). A number of "miniprimer sets" have been developed to amplify smaller portions of HV1 and HV2 (*145*, *151*).

A number of interlaboratory studies involving mtDNA sequencing have been conducted. A total of 64 laboratories from Spain, Portugal, and several Latin-American countries participated in a quality control exercise involving 5 samples (*152*). Ten laboratories participated in a study sponsored by the European DNA Profiling Group (EDNAP) that sequenced a total of 55 hairs (*153*). A collaborative exercise with the EDNAP mitochondrial DNA population database (EMPOP) examined the possible sources of error that arise during analysis and interpretation of mtDNA sequences (*154*). In this pilot collaborative study of 21 laboratories, 14 nonconcordant haplotypes (16 individuals errors) were observed out of a total of 150 submitted samples/haplotypes representing the examination of approximately 150 000 nucleotides (*154*). One study noted that regardless of the sequencing strategy used the biggest challenge in conducting mtDNA analysis remains in the efforts to reduce and eliminate errors in sequence review (*155*).

Concerns with mtDNA database sequence quality and the impact that it might have on accurately estimating frequency estimates for random matches have been raised (*156*, *157*). A call has been made for improved quality control in mtDNA used in forensic science (*158*). Artificial recombination in some mtDNA population databases was noted (*159*). The quality of the FBI's SWGDAM database was attacked (*160*). Several responses to this attack were made by the FBI (*161*−*163*). Using a statistical analysis tool called phylogenetics, the similarities and differences between multiple and closely related DNA sequences (i.e., from the same region) can be compared systematically (*144*). Phylogenetic measures were used to ensure high-quality mtDNA data from a Nairobi population study (*164*). Forensic population databases have been analyzed in terms of phylogenetic haplogroup information to aid in quality control of samples contained within a U.S. Caucasian (*165*), East Asian (*166*), and African American (*167*) population group. Simulations were performed to examine the effect of sample size on the number of different mtDNA haplotypes seen (*168*).

Over 1000 complete mtDNA genomes now exist in public DNA databases (*169*). A new method for mtDNA sequencing called polymorphism ratio sequencing was described that claims the capability to sequence the entire mtGenome in about 30 min using microchip separations (*170*). A total of 241 complete mtGenomes were sequenced from the 18 common European Caucasian HV1/HV2 types associated with mtDNA haplogroups H, J, T, V, and K (*171*). Examination of whole mtGenome sequence information expanded the 18 most common Caucasian HV1/HV2 types to 209 resolvable haplotypes (*171*). A set of 11 mtDNA polymorphisms was combined into a single multiplex assay to help resolve the most common Caucasian haplotype H (*172*). A multiplex SNP system for categorizing samples into 1 of the 9 major European Caucasian haplogroups using 16 coding region polymorphisms has been described (*173*). Another SNP typing assay reported the examination of 17 coding region SNPs in a single multiplexed detection assay (*174*). Sequencing of multiple individuals outside of the mtDNA control region was performed in mtDNA genes ATP6, ATP8, and ND4 (*175*) and between nucleotides 8306 and 9021 in the coding region (*176*). Efforts to infer the geographic origin of a mtDNA sequence profile were described (*177*).

Standard reference samples along with positive controls serve to demonstrate that mtDNA sequence analysis is being performed appropriately (*84*). The U.S. NIST has developed two Standard Reference Materials (SRMs) to aid in confirming sequencing results with mtDNA (*178*, *179*).

Some researchers are pursuing efforts to resolve mtDNA mixtures through cloning and sequencing the resulting HV1/HV2 regions from individual colonies (*180*). Denaturing HPLC has also been proposed as a possible approach to separating mtDNA amplicon mixtures (*181*, *182*) as has a mismatch primer-induced restriction site analysis method (*183*).

A reanalysis of the same samples used in the original study that was criticized for containing contamination due to an excessive number of amplification cycles (*184*, *185*) found a reduction

in the reported number of samples with heteroplasmic positions when a direct rather than a nested PCR approach was used (*186*). The ratio of bases at heteroplasmic sites may not stay the same across different tissues, such as blood and hair or between multiple hairs (*187*). Heteroplasmy among 480 hairs from 3 different individuals was examined with denaturing gradient gel electrophoresis (*188*). New efforts to monitor sequence heteroplasmy (*189*) and length heteroplasmy were described (*190*, *191*). There has been a single report published of the transmission of a paternal human mtDNA type in skeletal muscle (*192*). This paternal haplotype was not found in any other tissues though. Several additional studies with individuals having a similar muscle disease failed to find any evidence of paternal transmission of mtDNA (*193*−*195*). Concerns over the reliable recovery of the mtDNA from the Romanov remains were raised (*196*, *197*) and responded to by those involved in the original published work in 1994 (*198*) and 1996 (*199*).

Nonhuman DNA Typing Systems and Microbial Forensics. Synthetic DNA was mixed with printing inks to create a biometric DNA ink for authentication security (*200*). Sequence analysis of the mtDNA cytochrome *b* gene was shown to be effective at identifying the species of origin for a biological sample (*201*). A molecular genetic approach was published for forensic animal species identification using TP53 tumor suppressor gene amplification products (*202*). A panel of feline-specific STR markers dubbed the "MeowPlex" has been developed that contains 11 STRs on 9 different autosomes (*203*). In an initial study of 223 cats from 28 different breeds, the MeowPlex exhibited an average composite locus heterozygosity of 0.73 across the breeds (*204*). The power of discrimination with this 11plex feline STR multiplex ranged from 5.5×10^{-7} to 3.3×10^{-13} across the various breeds (*204*). A total of 15 canine STR loci have been characterized with sequenced alleles to define nomenclature for future work (*205*). These canine-specific STR markers were used to analyze dog bite wounds (*206*). Mitochondrial DNA sequencing was also performed on dog hairs (*207*). A call has also been made for standardization in dog mtDNA nomenclature (*208*).

Plant DNA can be important in cases involving common drugs such as marijuana. Efficient extraction protocols have been developed that yield 125−500 nanograms of DNA/100 mg of fresh plant tissue (*209*). Three techniques for performing marijuana DNA typing were reviewed (*210*). Even highly inbred individual plants can be distinguished with some techniques (*210*). Several STR markers have been recently reported for *Cannabis sativa* (*211*−*213*). A hexanucleotide repeat marker showed repeat units ranging from 3 to 40 in 108 tested marijuana samples, and primers amplifying this locus produced no cross-reactive amplicons from 20 other species of plants tested (*211*).

The U.S. efforts in building a response to bioterrorism were announced in a policy paper (*214*). The FBI has initiated a Scientific Working Group on Microbial Genetics and Forensics (SWGMGF) that will help develop guidelines related to the operation of microbial forensics (*215*). Currently there are an insufficient number of validated analytical tools to characterize and identify biological agents that might be used in a terrorist attack (*216*). Challenging attribution issues that confront microbial forensics have been discussed (*217*). Important requirements of biothreat detection assays are high sensitivity, high specificity in complex samples, fast measurement, compact design for portability and field use, and internal calibration and reference to ensure reliable results (*218*). Proficiency and validation testing are necessary to estimate false-positive and false-negative rates (*219*). As an example of the challenges faced in microbial forensics, it was pointed out that more than 125 000 samples were processed as part of the October 2001 anthrax case in the two years following this attack and yet no one has been charged with the crime to-date (*220*).

DNA Databases. International perspectives on forensic DNA databases have been described (*221*). Different criteria for entry of data into national DNA databases were summarized (*222*). The growth of DNA databases was reviewed (*223*). With limited budgets and difficult decisions being made by lawmakers on how best to prioritize funds to aid society, a business analysis of the expected return on an investment in forensic DNA technology has been described (*224*). For every dollar invested in forensic DNA testing, this analysis showed over $35 would be saved in terms of expense to victims and society (*224*). The latest developments in forensic DNA policy are available at http://www.dnaresource.com.

England's national DNA database maintains a 40% chance of obtaining a match between a crime scene profile and a "criminal justice" (arrestee or suspect) profile loaded into the database (*225*). It was noted that legacy data must be maintained through the continued use of the same STR markers or the value of the database will be diminished (*93*). In January 2003, the state of Virginia began collecting and analyzing DNA samples from all those *arrested* of certain violent crimes (*226*). Backlogs continue to grow as more and more samples are collected for input into DNA databases. Thousands of rape kits are sitting in police evidence rooms that are not submitted to crime laboratories (*227*).

Interpretation and Statistical Weight of DNA Typing Results. The primary issues and approaches to statistical interpretation of DNA evidence were reviewed (*228*). The effects of population structure and admixture were examined (*229*). Self-declaration of ethnicity can be a suitable method of categorizing samples on the basis of ethnicity (*230*). The observed and expected numbers of pairs of individuals with various numbers of matching or partially matching loci were evaluated in FBI and Australian databases (*231*). Allele sharing was compared between relatives and unrelated individuals (*232*). A Bayesian model was used to show how the potential for a false positive affects the evidentiary value of DNA evidence (*233*).

An analysis of 5700 profiles from 24 European populations that were generated with the SGM Plus kit loci was described along with a comparison of adjustment methods to test the robustness of a STR population database (*234*). Match probability formulas to calculate the effects of family relatedness that incorporate population substructure functions into them were developed (*235*). The specificity of sibship determination with 15 autosomal STRs generated using the Identifiler kit was examined (*236*). With STR analysis in paternity testing often examining a battery of a dozen or more loci, it is not uncommon to see two inconsistencies between a child and the true biological father (*237*). Mutation rates on 19 STR loci were described (*238*). Attempts to infer group membership were described with 100 Alu markers (*239*). Hardy-Weinberg equilibrium testing was used as a method for detecting

genotyping errors with 443 different SNP markers used to generate more than 107 000 genotypes (*240*).

Calculations for the assessment of DNA mixtures with the presence of relatives have been worked out (*241*). A computer program has been written to help in evaluating forensic DNA mixtures involving contributors from different ethnic origins (*242*). Probabilistic expert systems are also in development for aid in DNA mixture resolution (*243*). One forensic laboratory reported working 1547 criminal cases over a 4-year period that involved a total of 2424 samples, yet only 163 showed a mixed profile or 6.7% (*244*). Methods for estimating the potential numbers of contributors to an evidentiary stain based on a DNA profile were described (*245*).

Issues behind identification of mass disaster victims were examined with a special focus on the World Trade Center (WTC) victim identification efforts (*246*) and other terrorist attacks of September 11, 2001 (*145*). Experiences with performing DNA analysis on victims of the Swiss Air Flight 111 airline crash (*247*) and the WTC terrorist attacks (*248*) were described.

General Reviews. The journal *Nature Genetics Reviews* has published nice reviews on microsatellites (*55*), Y-chromosome analysis (*105*), and forensic DNA analysis (*249*). The business case for forensic DNA analysis along with a calculation of a return on investment was described (*224*). A bibliometric analysis of 14 210 forensic science and legal medicine publications between 1981 and 2003 was performed (*250*).

TRACE EVIDENCE

Pertroleum Products and Explosives. Preferential extraction of aliphatic and aromatic hydrocarbons from fire debris samples was accomplished by solid-phase microextraction (SPME) (*251*). GC/MS analysis of ignitable liquids was reported using solvent-desorbed SPME (*252*). Classification of premium and regular gasoline by GC/MS, principal component analysis, and artificial neural networks has been reported (*253*). Chemical fingerprinting of unevaporated automotive gasoline samples was studied using a combination of SPME and GC/MS (*254*). GC/MS using selected ion monitoring was used to discriminate unevaporated and evaporated samples of gasoline (*255*, *256*). GC/MS was used to compare gasoline samples obtained from different sources based on the difference in amounts of certain components found in the headspace of gasoline using target response data (*257*). The aromatic content in various medium-range distillate products has been determined (*258*). Accelerant identification in fire debris by thermal desorption cold trap injection-GC/MS has been reported (*259*). The effectiveness of sampling highly volatile accelerants at the fire scene using a portable air pump has been examined (*260*). Background and pyrolysis products that may interfere with the forensic analysis of fire debris have been characterized (*261*). Transfer and persistence studies were undertaken to investigate the potential transfer and persistence of gasoline onto vehicle carpets through the normal usage of motor vehicles (*262*). The evaluation of the extent of transporting or "tracking" an identifiable ignitable liquid (gasoline) throughout fire scenes during the investigative process has been reported (*263*). Recent advances in the applications of forensic science to fire debris analysis has been reviewed (*264*). The mechanisms of pyrolysis relating to to fire debris analysis has been reviewed (*265*).

Different sampling materials were tested for the detection of 1,3,5-trinitro-1,3,5-triazacyclohexane (RDX), Pentaerythritol tetranitrate (PETN) and cyclotetramethylene-tetranitramine (HMX) explosives by using ion mobility spectrometry (*266*). Solid-phase desorption/gas chromatography/ion mobility spectrometry of RDX and HMX explosives was conducted by using Teflon disks and filter paper as sampling materials (*267*). Ion mobility spectrometry analyses of triacetonetriperoxide, ammonium nitrate, black powder, and smokeless powder were conducted to establish peak parameters and detection limits for these explosives (*268*). The capability of secondary electrospray ionization in combination with ion mobility spectrometry to detect RDX, nitroglycerin, and PETN has been explored (*269*). Triacetone triperoxide (TATP) has been detected by ion mobility spectrometry (*270*). TATP, diacetone diperoxide, and hexamethylene triperoxide diamine are sensitive and powerful organic peroxide explosives. Their forensic characteristics have been reviewed (*271*). A reversed-phase HPLC method for peroxide-based explosives has been described (*272*). Trace analysis of peroxide explosives by HPLC-atmospheric pressure chemical ionization-tandem mass spectrometry for forensic applications has been investigated (*273*). The recovery of TATP by SPME was compared with headspace and with adsorption on Amberlite XAD-7 (*274*). Trace explosive detection in aqueous samples by SPME in combination with ion mobility spectrometry has been examined (*275*). SPME coupled with on-column GC and electron capture detection has been examined for the postblast analysis of organic explosives (*276*). Various factors governing the detectability of explosive traces after being soaked in water were studied (*277*). Liquid chromatography/mass spectrometric analysis of RDX adduct ions has been reported (*278*). The quantification and aging of the postblast residue of TNT landmines has been studied (*279*). The separation of explosives by capillary electrophoresis was optimized with the aid of artificial neural networks (*280*). Electrospray mass spectrometry when used with spectral libraries has been demonstrated to be a useful method for rapid identification of inorganic oxidizers commonly present in commercial and improvised explosives (*281*). SPME followed by GC/MS has been applied to the analysis of ammonium nitrate-based explosives (*282*). The sorption of explosives TNT, RDX, PETN, and TATP to hair during exposure to their vapors has been examined (*283*). GC/MS has been employed for the analysis of organic explosives using electon impact and negative ion chemical ionization (*284*). The rapid screening of various high-grade explosives by HPLC with monolithic stationary phases has been investigated (*285*). A highly sensitive screening method based on HPLC-atmosphere pressure ionization-mass spectrometry has been developed for the analysis of 21 nitroaromatic, nitramine and nitrate ester explosives (*286*). A study was conducted in an attempt to develop a metallographic method for the investigation of pipe bombings (*287*). A study was undertaken to assign a rough order of magnitude for the amount of explosive residue likely to be available in real-world searches for clandestine explosives (*288*). A survey was carried out in order to determine the background levels of explosives traces in public places (*289*). A photoluminescence-based field method for detecting traces of explosives has been studied (*290*).

Hairs, Fibers, Glass, and Paint. A study was conducted to investigate the accuracy between two methods of hair analysis PCR-STR DNA analysis and microscopic comparison analysis (*291*). The persistence on clothing of human scalp hairs under a number of different circumstances has been explored (*292*). The current state of forensic hair examination has been reveiwed (*293*).

Textile fibers recovered from one item of evidence from each of 20 unrelated crimes in three categories (bank robbery, kidnapping, and homicide) were cross-compared. No two fibers were found to exhibit the same microscopic characteristics and analytical properties (*294*). Samples of orange and green cotton fibers were examined using UV−visible microspectrometry (*295*). LC/MS has been used for the identification of dyes extracted from textile fibers (*296*). A study was undertaken to examine the random distribution of fibers on clothing (*297*). Forensic analysis of poly(ethylene terephthalate) fibers by infrared spectroscopy has been studied (*298*). The Maxcan fiber finder system has been developed for the primary purpose of searching for fibers on tape lifts. The ability of the Maxcan system to search for different fiber types and colors under varying conditions has been studied (*299*). The history of the European Fibers Group to include its aims and achievements from the time of its formation in 1993 to the present day has been reviewed (*300*). A population study of fibers in human hair has been carried out (*301*). Inductively coupled plasma mass spectrometry (ICPMS) was used to analyze glass evidence (*302*). Analysis and comparison of glass fragments by laser ablation-inductively coupled plasma-mass spectrometry and ICPMS has been undertaken. The laser ablation method was simpler, faster, and offered an excellent alternative to analysis of glass in forensic casework samples (*303*). The effect of fractionation on the forensic elemental analysis of glass using laser ablation inductively coupled plasma mass spectrometry has been studied (*304*). The classification and discrimination of glass fragments has been undertaken using energy-dispersive X-ray fluorescence (*305*). The spatial variation of refractive index in a pane of float glass was examined (*306*). A study was made of the probable distributions of refractive indices of the recovered glass given a range of casework type scenarios involving breakage and backscatter (*307*).

A study was undertaken to assess the distinctiveness of automotive paints (*308*). Red spray paints were characterized by optical microscopy, Fourier transform infrared spectroscopy, and X-ray fluorescence (*309*). A market study of 40 different green spray paints was carried out using FT-IR and Raman spectroscopy (*310*). Trace elemental analysis of automotive paints by laser ablation-inductively coupled plasma-mass spectrometry has been reported (*311*). Trace elemental analysis of glass and paint samples of forensic interest by ICPMS using laser ablation solid sample introduction was reported (*312*).

Gunshot Residues. Laser-induced breakdown spectroscopy was used to detect gunshot residue (GSR) on a shooter's hand (*313*). An attempt was made to build a classification scheme for GSR samples originating from four types of ammunition collected from shooters' hands immediately after shooting (*314*). A novel method for extraction and analysis of gunpowder residues on double-side adhesive-coated stubs has been reported (*315*). A novel collection method for gunshot residues using a sampling procedure based on ethylenediaminetetraacetic acid solution as a complexing agent on moistened swabs has been reported (*316*). Experiments were conducted to collect gunpowder residues from shooters' clothing by vacuum and to analyze them by GC./thermal energy analyzer, ion mobility spectrometry, and GC/MS (*317*). A proficiency test on the detection and identification of GSR by SEM/EDX was organized and performed (*318*). A protocol has been presented for the collection and analysis of gunshot residues from hair (*319*). An X-ray mapping technique has been investigated for the purpose of discriminating gunshot residue particles from other nonfirearm lead−antimony−barium aggregates (*320*, *321*). A previously unreported type of GSR particle consisting of glass fused with other primer components has been reported (*322*). Pyrotechnic reaction residue particles have been characterized by SEM/EDS (*323*).

Organic components of smokeless gunpowders have been analyzed by HPLC and micellar electrokinetic capillary chromatography (*324*). Methodology was reported to detect different formulations of smokeless powders by ion mobility spectrometry (*325*). A gradient reversed-phase liquid chromatography-electrospray ionization-mass spectrometric method was developed to determine compositional variation in the organic additives of smokeless powders (*326*).

The estimation of time since the latest discharge of pistols and revolvers has been achieved by a SPME sampling technique (*327*). A technique for the estimation of time since discharge of a given class of spent shotgun shells is presented. The technique involved the use of SPME sampling from the atmosphere inside spent shotgun shells (*328*). A sensitive method for the identification of residual levels of ascorbic acid in black powder substitutes has been presented (*329*). The velocity of over 800 gunshot residue particles from 8 different sources was determined using high-speed stroboscopic photography (*330*). A review of recent developments in the methods used for the chemical analysis. in investigations of firearm-related events is provided (*331*).

Fingerprints. Exogenous and individual factors affecting DNA typing from latent fingerprints have been examined (*332*). Modifications have been reported for the silver physical developer of latent fingerprints (*333*). Latent fingerprint detection using visible absorption and luminescence has been demonstrated (*334*). 1,2-Indandione has been evaluated as a reagent for fingerprint detection (*335*). Latent fingerprint deposits have been characterized by infrared microspectroscopy (*336*). Advances in the silver physical development of latent prints on paper have been described (*337*). Genipin, the hydrolytic product of geniposide, which is extracted from gardenia fruit, has shown good potential as a fingerprint reagent (*338*). The performance of two spray reagents, iodine−benzoflavone and ruthenium tetroxide, was evaluated for the detection of latent fingerprints (*339*). The influence of humidification in the ESDA process on subsequent development of fingerprints on paper items was studied (*340*). A study was devised to examine the effects of a latent print development technique (ninhydrin, physical developer, and bleach enhancer) on the physical and chemical examination of documents produced from copiers and printers (*341*). Image enhancement methods to improve the legibility of digital images such as fingerprints and vehicle plate numbers have been applied (*342*). A method to produce easy visualization of fingerprints on different

types of plastic tape, including black electrical tape, without the need for expensive equipment has been studied. The methods selected were to sublime disperse dyes into the tape, both with and without the fuming of cyanoacrylate, everywhere except for where the fingerprint was applied (*343*). In an attempt to maximize the yield of latent fingerprints from paper items, a study of a fundamental process between fingerprint deposits and paper was conducted (*344*).

Miscellaneous. A method was developed to screen for pepper spray residue using GC, TLC, and diffuse reflectance IR Fourier transform spectroscopy to screen for dried pepper spray stains (*345*). LC/MS has been applied to the analysis of pepper sprays (*346*). A study to investigate the evidential value of blue gel pen inks has been undertaken (*347*). Photodegradation and laser desorption mass spectrometry was used for the characterization of dyes used in red pen inks (*348*). Results show that it is possible under appropriate conditions to determine the relative age of entries written by ballpoint pen within a relatively short time scale (*349*). Surface-enhanced Raman scattering spectrosocopy was used to examine inks (*350*). HPLC was used to identify the decomposition products of ball-point ink components as they age (*351*). The aging process of some inks was studied to evaluate whether it is possible to date them (*352*). Multivariate chemometrics for the discrimination of blue ball-point inks based on their visible spectra has been evaluated (*353*). The identification of 2-phenoxyethanol in ball-point inks using GC/MS has been reported (*354*). Black gel inks wee differentiated using microscopy, visible and near-infrared reflectance, near-infrared luminescence, thin-layer chromatography, spot tests, and GC/MS (*355*). SPME/GC/MS was used to characterize the volatile components associated with U.S. currency, U.S. currency inks, and Canadian currency (*356*). The direct detection and identification of staining dyes from security inks, in the presence of other colorants, on currency and fabrics by laser desorption mass spectrometry has been reported (*357*).

Copy toner samples were analyzed using SEM/EDX and pyrolysis GC/MS (*358*). Microscopical reflection−absorption by IR spectroscopy was shown as a viable technique for analyzing the polymer resins contained in dry, black photocopy and printer toners (*359*). Copy toner samples were analyzed using reflection−absorption IR microscopy (*360*). Identification of colorless, transparent, pressure-sensitive adhesive polypropylene tape was performed using IR and pyrolysis GC/MS (*361*). The use of SPME combined with GC to identify the signature odors that law enforcement-certified detector dogs alert to when searching for drugs, explosives, and humans has been studied (*362*). Spectral enhancement of leucocrystal violet-treated footwear impression evidence in blood has been studied (*363*). Analysis of a contaminated brake fluid revealed the contaminant to be power steering fluid (*364*). The characterization of condom lubricant components using Raman spectroscopy and Raman chemical imaging has been reported (*365*). Traces of a barrier/spermicide, nonoxynol-9, in a matrix consisting of approximately two parts poly(ethylene glycol) (PEG)1000 to one part PEG 1450 has been detected (*366*). The evidential value of cosmetic foundation smears in forensic casework has been investigated (*367*). SEM/EDS analysis has been applied to the discrimination of forensic soil (*368*). A repeatability test, comparing conventional X-ray diffraction analyses with the technique of quantitative X-ray diffraction as a determinant of mineral abundance has been examined (*369*). Tires and tire traces have been analyzed by FT-IR and pyrolysis-GC/MS (*370*). Quantitative data have been reported on the amounts of iron that are transferred to the hands upon holding a firearm (*371*). SEM/EDX as a tool for forensic examinations has been reviewed (*372*). ICPMS trace element analysis as a forensic tool has been reviewed (*373*). Use of X-rays in the United Kingdom forensic science service has been reviewed (*374*). Applications of mass spectrometry in forensic science have been reviewed (*375*). The potential of thermogravimetric analysis as a forensic tool for determination of different species of polymers has been studied (*376*). A quick and easy method for impregnating nylon transfer membranes with leucocrystal violet for the purpose of lifting and enhancing impressions made in blood has been reported (*377*).

DRUGS AND POISONS

Ethanol and Volatiles. A review on detection of ethanol in blood, determination of breath ethanol, pharmacokinetics of ethanol, and calculations of the ethanol concentration has been published (*378*). A study of the effectiveness of the built-in acetone correction procedure, in measuring blood alcohol concentration (BAC), using the Intoxilyzer 5000C has been reported (*379*). The precision of breath alcohol concentrations (BrAC) measured with the Draeger Alcotest 7110 evidential breath tester was compared with the precision of BACs obtained by forensic means (*380*). Effects of eight possible interfering compounds on ethanol analysis were determined in a procedure simulating a human breathing on a Drager 7110 MK III FIN evidentrial breath analyzer (*381*). Breath alcohol measurement variability associated with different instrumentation and protocols has been discussed (*382*). The extent of alcohol depletion occurring in simulator solutions used in performing repeated calibration checking of an Intoxilyzer 5000C instrument operated in recirculation mode has been studied (*383*). The calibration factor in BrAC has been discussed (*384*). A laboratory experiment that uses an FT-IR spectrometer to measure BrACs has been described (*385*). The population pharmacokinetics of ethanol in drinking drivers was studied by measuring BrAC using a three-wavelength infrared spectrophotometer (*386*). The effect of breath freshener strips on two types of breath alcohol testing instruments has been studied (*387*). The mouth alcohol effect was found to be substantially decreased by increasing the salivary flow rate through testing with the Intoxilyzer 5000C (*388*). Retrospective studies were conducted of drinking drivers who were arrested with the highest Intoxilyzer 5000C results in the city of Toronto between 1995 and 1999 (*389*, *390*). A breath alcohol sensor using a copper electrode in an alkaline medium has been developed (*391*). Ethanol concentrations in venous blood and end-expired breath have been compared during a controlled drinking study (*392*). Comparative regression analysis of concurrent elimination-phase blood and breath alcohol concentration measurements to determine hourly degradation rates has been reported (*393*). A sensitive and specific method using static headspace GC/MS has been developed for quantitative determination of ethanol in biological fluids using 1-propanol as internal standard (*394*). The determination of ethanol in human blood and urine by automated headspace (HS)-SPME and capillary GC has been described (*395*). The salting-out effects in forensic blood alcohol determination has been studied using semiauto-

mated HS-GC (*396*). The uncertainty for the measurement of ethanol in blood by HS-GC has been discussed (*397*). Urine/blood ratios of ethanol in deaths attributed to acute alcohol poisoning and chronic alcoholism have been reported (*398*, *399*). Serum alcohol concentrations in trauma patients were determined by immunoassay and GC (*400*). The effectiveness of sodium fluoride has been studied at various storage temperatures for the prevention of microbial ethanol formation (*401*). Handwriting changes under the effect of alcohol have been studied (*402*). The pharmacokinetics of ethanol in saliva and blood was compared according to gender (*403*). Studies have shown that vitreous humor may be a reliable matrix for ethanol analysis following prolonged refrigerated storage of the samples (*404*). Ethanol was measured in blood and urine by HS-GC, and ethylglucuronide was determined in urine by LC/MS (*405*). BAC was determined by GC-flame ionization detection (FID), drugs of abuse were determined by GC/MS, and other drugs were determined by HPLC with diode-array detection (DAD) in the blood of 900 drivers involved in nonfatal accidents and emergency patients (*406*).

Tissue distribution of trichloroethylene was determined by static headspace GC/MS in a case of accidental acute intoxication by inhalation (*407*). Stability of toluene and reduction of acetone to 2-propanol in homogenates of the human liver, brain, and lungs has been studied (*408*). Concentrations of toluene in the body of a victim killed by an injury to the head shortly after ingesting thinner have been reported (*409*). Biological fluids and organs were collected and analyzed for gas components by HS-GC (*410*). A new method for the determination of %COHb by GC-thermal conductivity detection that incorporates sodium dithionite has been developed (*411*). Gas samples were analyzed by headspace GC/MS for the analysis of argon in biological fluids from a suffocation victim (*412*). Phenol and *o*-cresol were determined by GC/MS in postmortem bioliogical fluids from a fatal poisoning victim (*413*). Formic acid was analyzed in blood and tissues from acute methanol intoxication subjects by headspace GC-FID (*414*).

Cannabinoids. Constituents in cannabis preparations have been quantitatively determined by high-resolution GC-FID and HPLC/UV detection (*415*) and planar chromatographic methods such as automated multiple development and optimum performance laminar chromatographic techniques (*416*). Randomly collected paper currency notes from the United States and four foreign countries were analyzed for cannabis (marijuana) constituent residues by washing the individual bills, extraction using liquid−liquid extraction, derivatization, and quantitation by GC/MS using selected ion monitoring (SIM) (*417*). The potency of cannabis was determined by analyzing Δ^9-tetrahydrocannabinol (THC) with GC using an external standard (*418*). Microsatellite markers were developed for *Cannabis sativa* L. (marijuana) to be used for DNA typing (genotype identification) and to measure the genetic relationships between the different plants (*419*).

A simple and reliable liquid−liquid extraction method for the deterimination of THC in whole blood utilizing GC/MS has been described (*420*). The acute and residual cognitive effects of THC in infrequent cannabis users have been studied (*421*). Rapid and sensitive methods for the simultaneous confirmatory analysis of THC, 11-hydroxy-Δ^9-tetrahydrocannabinol and 11-nor-9-carboxy-DELTA^9-tetrahydrocannabinol (THC-COOH) in human plasma have been developed and fully validated by means of HPLC/tandem mass spectrometry (LC/MS/MS) (*422*) and using hydrolysis, solid-phase extaction (SPE), and positive chemical ionization (PCI) GC/MS (*423*).

THC-COOH was analyzed in urine by GC/MS (*424*), SIM GC/MS (*425*, *426*) immunoassay and GC/MS (*427*), and LC/MS/MS (*428*). THC-COOH and creatinine ratios in urine were studied by analyzing THC-COOH by GC/MS and by analyzing creatinine as an index of dilution (*429*−*431*). THC was analyzed in oral fluid by enzyme immunoassay (EIA) GC/MS/MS (*432*) and radioimmunoassay (RIA) and GC/MS (*433*). Cannabinoids have been detected in hair using alkaline hydrolysis, liquid−liquid extraction, and GC/MS (*434*), automated alkaline hydrolysis, and HS-SPME followed by on-fiber derivatization and GC/MS (*435*, *436*), immunoassay and GC/MS/MS (*437*) and LC/MS (*438*). The effect of hair color on the concentration of THC in human hair has been evaluated (*439*).

Morphine and Related Narcotics. Heroin was detected by GC/MS (*440*), HPLC (*441*) and high-performance thin-layer chromatography (HPTLC) (*442*). The main components of heroin were analyzed by GC and GC/MS (*443*). Heroin and its impurities have been analyzed by capillary electrophoresis (CE) (*444*) and GC-FID (*445*). Procaine hydrochloride was identified as a heroin diluent using one- and two-dimensional (1D and 2D) proton and carbon-13 NMR (*446*). Mannitol hexaacetate has been detected by GC/MS in some brown illicit drug seizures in which diacetylmorphine was completely lacking (*447*). Different comparison methods have been tested and a method based on a correlation coefficient using a square cosine function was chosen to compare heroin chemical profiles (*448*). Heroin samples were analyzed for trace elements by neutron activation analysis (*449*). The microflora of heroin samples has been reported (*450*).

A general overview of the electrochemical methods used for detection and quantification of opiates in a variety of matrixes has been reported (*451*). A rapid and sensitive method to determine morphine in street opium samples has been reported which uses thermal desorption GC and a microfurnace pyrolyzer (*452*). Inductively coupled plasma-optical emission spectroscopy was used for multielement analysis of Indian gum opium (*453*).

Opioids have been determined in blood by GC/MS (*454*, *455*). A rapid and sensitive method for quantification of morphine and morphine glucuronides in human plasma using HPLC with electrospray ionization MS was developed (*456*). A simple SPE assay for the HPLC-DAD or HPLC/UV analysis of morphine and its glucuronides in serum samples has been reported (*457*). Heroin metabolites including morphine have been detected in urine by latex agglutination inhibition reaction (*458*), immunoassay and GC/MS (*459*), EIA (*460*), immunoassay using a surface plasma resonance-based biosensor (*461*), automated extraction procedure and GC/MS (*462*), and CZE/UV (*463*). The postmortem distribution of heroin metabolites in femoral blood, liver, cerebrospinal fluid, and vitreous humor has been reported (*464*). An extraction procedure for detecting low concentration levels of 6-MAM from urine with a mixed-mode SPE sorbent has been described (*465*). Comparisons of hydrolysis using two different sources of β-glucuronidases and various concentrations of hydrochloric acid were made to detect codeine and morphine in urine using solid-phase extraction, derivatization, and quantification by SIM using GC/MS (*466*). Methods to detect codeine and morphine in urine, other biological fluids, and hair after poppy seed consumption have been reported (*467*−*471*). The accuracy of screening postmortem whole blood for oxycodone using the

ratio of the oxycodone immunoassay response to the response for the specimen obtained with a general opiate-class immunoassay has been reported (*472*). Postmortem oxycodone and hydrocodone blood concentrations have been reported (*473*). Acetylcodeine has been detected by GC/MS as a urinary marker to differentiate the use of street heroin and pharmaceutical heroin (*474*). Reticuline was detected by GC/MS and GC/CIMS in opium and the urine of opium users (*475*).

Hair and other narcotics have been detected in hair using SPE after digestion followed by GC/MS (*476*, *477*), SIM using GC/MS (*478*, *479*), immunoassay and GC/MS (*480*), and CE (*481*). The evaluation of external heroin contamination and risk of false positives has been reported (*482*). Changes in the chemical structures of morphine and codeine in the presence of hydrogen peroxide were examined with LC/MS (*483*). The effect of hair color on the incorporation of codeine into human hair has been reported (*484*).

Opiates have been determined in oral fluids by immunoassay and GC/MS (*485*), immunoassay and GC/MS (*486*), SPE and GC/MS (*487*), and immunoassay and GC/MS/MS (*488*). The simultaneous detection and quantitation of morphine, 6-acetylmorphine, and cocaine in toe nail and hair samples has been described using liquid−liquid and solid-phase extraction techniques followed by deriviatization and SIM-GC/MS (*489*). Heroin was determined after embalmment (*490*).

Cocaine. Studies on spot tests and microcrystalline tests for the identification of cocaine have been reported (*491*). A study of acids used for the acidified cobalt thiocyanate test for cocaine base has been reported (*492*). Cocaine has been detected by piezoelectric immunosensor (*493*), Raman spectroscopy using Kerr gated fluorescence rejection (*494*), and square-wave voltammetry (*495*). A capillary electrophoretic method for the separation of cocaine and its stereoisomers was developed (*496*). A laboratory activity that teaches experimental design using GC/MS analysis of cocaine in money has been described (*497*). Analysis of banknotes via counting machines detected cocaine (*498*). The identification of phenacetin and cocaine from packages in the stomach of a body packer was performed by GC/MS (*499*).

Cocaine and metabolites have been detected in blood by column-switching HPLC/UV (*500*), GC/MS (*501*), GC/MS, LC/ESI-MS/MS, and liquid chromatography/atmospheric pressure chemical ionization-tandem mass spectrometry (LC/APCI-MS/MS) (*502*), SPE followed by GC-FID (*503*), ELISA, GC/MS, and GC/NPD (*504*), and LC/MS/MS (*505*). The pyrolysis product anhydroecgonine methyl ester was detected after cocaine smoking by GC/MS (*506*). The correlation between pharmacological effects and plasma cocaine concentrations after smoke administration has been reported (*507*).

Urinary elimination of cocaine metabolites has been reported (*508*). The prediction of impairment from urine benzoylecgonine concentrations has been reviewed (*509*). The deterimination of cocaine and metabolites in human hair have been reported using HPLC/DAD (*510*), HPLC (*511*), SPE and GC/MS in the SIM mode (*512*), SPE and LC/MS/MS (*513*), and GC/MS (*514*). Cocaine and metabolite levels were determined in the hair of 75 subjects who had produced cocaine-positive urine results (*515*). The determination of cocaine and its major metabolites in saliva was developed using liquid−liquid extraction followed by derivatization and GC/MS (*516*). A comparison of analysis of sweat patches for cocaine and metabolites by GC/PICIMS and RIA has been reported (*517*). The detection of crack and other cocaine use has been reported by analysis of sweat patches using EMIT and GC/MS (*518*). A comparison of urine, skin swabs, and sweat patches for the detection of drugs, including cocaine, has been reported using immunoassays and GC/MS (*519*). Occupational cocaine exposure of crime laboratory personnel has been studied (*520*). A LC/MS method for the determination of opiates and cocaine in meconium has been described (*521*). Cocaine analytes and opiates have been detected in postmortem nail samples by GC/MS (*522*). Cocaine and metabolites have been detected in fixed liver tissue and formulin solutions (*523*).

Amphetamines. Amphetamine and related substances have been analyzed by CZE (*524*) and derivatization followed by GC/MS (*525*). Chromatographic (*526*) and chromatographic and spectroscopic (*527*) methods of identification for the side-chain regioisomers of 3,4-methylenedioxyphenethylamines (MDMA). The risk for false-positive identification of phenmetrazine in an ephedrine-containing sample analyzed by GC/MS has been described (*528*). A review of the techniques used to analyze Ecstasy samples has been published (*529*). Methamphetamine impurity profiling has been accomplished by capillary column GC-FID (*530*) and GC/MS and GC-FID (*531*). Profiling of impurities in *p*-methoxymethamphetamine (PMMA) by means of SPE/TLC has been reported (*532*, *533*). Chromatographic and mass spectral studies on isobaric and isomeric substances related to MDMA have been reported (*534*). Ecstasy tablets have been analyzed using near-IR spectroscopy (NIR) in diffuse reflectance and in transmission mode (*535*), Raman spectroscopy (*536*), GC/MS and HPLC (*537*, *538*), gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) (*539*, *540*), CE (*541*), capillary GC (*542*), HPLC and ESI-MS (*543*), and GC/MS (*544*−*546*). The analysis of dyes in illicit pills containing amphetamine and derivatives were analyzed by SPE, TLC, and CZE-DAD (*547*). The synthetic origin of amphetamines was established by ^{2}H NMR spectroscopy (*548*). A rapid and simple GC/MS screening method for 4-methoxyphenol in illicitly prepared PMA has been described (*549*). The origin of ephedrine used as a precursor for illicit methamphetamine has been determined by stable isotope ratio mass spectrometry (IR-MS) (*550*). The identification of d−*N*,*N*-dimethylamphetamine (DMA) was established by GC/MS, FTIR, HPLC, mp, and Optical Rotation (*551*). The analysis of 5-methoxy-alpha-methyltryptamine (5-MeO-AMT) via color testing and GC/MS has been presented (*552*). Electron ionization mass spectrometry has been used as a tool for the investigation of the ortho effect in fragmentation of some Schiff bases derived from amphetamine analogues (*553*). Chromatographic methods of the analysis of amphetamine-type drugs have been reviewed (*554*). LC has analyzed amphetamine enantiomers after derivatization (*555*, *556*) and CE-ESI/MS (*557*). A chromoreactand for optical sensing of amphetamines has been described (*558*). Analysis of red phosphorus in clandestine methamphetamine has been reported using pyrolysis GC/MS (*559*). Anise oil was investigated has a *p*-methoxyamphetamine (PMA) using GC/MS and GC/HS-SPME/MS (*560*).

Amphetamine and similar drugs have been detected in blood by GC/electron capture detection (GC/ECD) (*561*), GC/NICI-MS (*562*), ELISA (*563*), LC/MS (*564*), GC/MS and HPLC/MS (*565*), GC/MS (*566*), GC/NPD and GC/MS (*567*), and headspace capillary GC with large volume injection (*568*). A method for the quantitative enantioselective analysis of amphetamine in human

plasma by GC/NICI-MS has been presented (*569*). A sensitive and specific HPLC-APCI-MS method for the detection of selegiline and its metabolites, including methamphetamine and amphetamine, in human plasma has been described (*570*). Amphetamines have been analyzed in urine by a miniaturized sample preaparation method, on-column derivatization and GC/MS (*571*), immunoassays (*572*, *573*), HPLC with fluorescence detection (*574*), GC/MS-SIM (*575*), immunoassay, HPLC, and GC/MS (*576*), immunoassay, SPE, and GC/MS (*577*), immunoassay and GC/MS (*578*), on-column derivatization and GC/MS (*579*), liquid−liquid extraction, derivatization and GC/MS (*580*), surface-activated chemical ionization ion trap mass spectrometry (SACIMS) (*581*), fluorescence polarization immunoassay (FPI) and GC/MS (*582*), HS-SPME and GC/MS (*583*), SPE and LC/MS, LC−UV, and GC/NPD (*584*), HS-SPME and GC-FID (*585*), GC/PIMS (*586*), SPE and HPLC−UV (*587*), SPME and HPLC (*588*), and LC/MS/MS (*589*).

The enatiomeric determination of amphtetamine and related compounds in urine has been reported using CE (*590*−*592*), HPLC (*593*), CE/MS (*594*), GC after precolumn derivatization (*595*), and derivatization and GC/MS (*596*). Amphetamine enantiomer excretion profile following administration of alderall has been studied (*597*). The analysis of methamphetamine and its metabolites in hair have been reviewed (*598*). The analysis of amphetamines, related compounds, and metabolites in hair has been reported using HPLC with fluorescence detection (*599*−*601*), GC/MS (*602*, *603*), HPLC-APCI-MS (*604*), RIA and LC/MS/MS (*605*), and immunoassay (*606*). Deposition characteristics of methamphetamine and amphetamine in fingernail clippings and hair sections have been studied by GC/MS (*607*). MDMA has been detected in sweat by immunoassay and GC/MS (*608*). Tissue concentrations (*609*) and blood levels (*610*) of MDMA and its metabolite have been reported. A review on the identification and quantification of 4-bromo-2,5-dimethoxyphenethylamine has been published (*611*). Methods for the extraction, identification, and quantitation of PMA and PMMA have been described (*612*). A neolignan-type impurity arising from the peracid oxidation reaction of anethole in the surreptitious synthesis of PMA has been reported (*613*). The effectiveness of multiple internal standards in the mass spectrometric analysis of MDMA and related compounds has been studied (*614*). The pyrolysis products of methamphetamine have been studied by GC/MS and LC/ESI-MS (*615*).

Benzodiazepines. Diazepam has been determined in cream biscuits by HPLC (*616*) and HPTLC and GC/MS (*617*). Benzodiazepines have been detected in biological fluids by LC/APCI-MS (*618*), LC/MS-ion trap (*619*), ELISA (*620*), ion trap GC/MS (*621*), capillary GC (*622*), HPLC (*623*), HPLC/APCI-MS/MS (*624*), NCI/GC/MS (*625*), HPLC/DAD/MSD (*626*), and immunoassay and GC/MS (*627*). The plasma-to-whole blood distribution of diazepam and nordiazepam was investigated (*628*). The relationship between benzodiazepine concentration and simple clinical tests for impairment of drugged drivers has been studied (*629*). Benzodiazepines have been detected in hair and other biological fluids using LC/MS/MS (*630*−*632*), TOF-SIMS (*633*), and NCI/GC/MS (*634*). Positive lorazepam drug-impaired driving cases have been reviewed (*635*). The distribution of nitrazepam and 7-aminonitrazepam (*636*) and tramadol (*637*) in the biological fluids of poisoning victims has been reported.

γ-Hydroxybutyrate. γ-hydroxybutyrate (GHB) and related compounds have been identified by derivatization and GC/MS (*638*, *639*), proton and carbon NMR spectroscopy (^{1}H and ^{13}C NMR) (*640*), liquid−liquid extraction and infrared spectroscopy (*641*), and osmolality (*642*). A comprehensive analytical profile for 1,4-butanediol has been presented including GC/MS, FT-IR, NMR, GC-IRD, and GC-FID (*643*). The pharmacology and toxicology of GHB has been reviewed (*644*). A reliable, sensitive, rapid, and quantitative enzyme-based assay for GHB in alcoholic beverages has been developed (*645*).

GHB has been detected in blood and other biological fluids by HS-GC (*646*, *647*), CZE/UV (*648*), GC/MS (*649*−*653*), GC-FID and GC/MS (*654*, *655*), PCI-GC/MS (*656*), SPE and GC/MS (*657*), and CZE/UV and ESI-ITMS (*658*, *659*). GHB has been detected in hair and other biological fluids by GC/MS/MS (*660*).

Miscellaneous. LSD and related drugs have been analyzed by GC/ITMS (*661*) and sweeping-micellar electrokinetic chromatography (MEKC) (*662*). LSD and related drugs have been analyzed in urine and blood by LC/MS/MS (*663*), sweeping-MEKC, and cation-selective exhaustive injection-sweep-MEKC (*664*), GC/ITMS (*665*), SPE and LC/MS (*666*), and GC/MS (*667*). An analytical perspective on favored synthetic routes to the psychoactive tryptamines was presented (*668*). Dimethyltryptamine was identified by GC/MS (*669*). Barbiturates have been analyzed in biological fluids and tissues by micellular liquid chromatography (*670*), high-speed analytical countercurrent chromatography, and HPLC/APCI (*671*), capillary electrochromatography (*672*), SPME-GC/MS (*673*), and SPME-GS/MS/MS (*674*). The distribution of butalbital in postmortem tissues and fluids from nonoverdose cases has been reported (*675*).

Methadone and its role in drug-related fatalities have been reported (*676*, *677*). Chiral separation of methadone and related drugs has been described using CE (*678*) and LC/MS (*679*). Methadone was analyzed in hair by GC/MS-SIM (*680*). Fentanyl has been identified in biological fluids by SPE and GC/MS (*681*). A GC/MS method for the quantification of *p*-fluorofentanyl in powder has been developed (*682*). DNA profiling of mushrooms containing psilocin and psilocybin has been reported (*683*, *684*). Mushrooms have been analyzed for the identification of psilocin by TLC and GC/MS (*685*) and HPLC/UV (*686*). Psilocin was detected in urine by LC/MS and LC/MS/MS (*687*). FT-IR spectroscopy using a diamond composite ATR crystal and NIR-FT-Raman spectroscopy techniques were applied for the simultaneous identification and quantification of the most important alkaloids in poppy capsules (*688*). The analysis and distribution of mescaline in postmortem tissues has been determined by GC/MS and GC/NPD (*689*). A method comprising SPE and HPLC was developed for the detection of atropine and scopolamine (*690*). Analysis of crude extracts of khat (*Catha edulis*) was done by LC/MS (*691*). A method for the quantitation of of khat constituents in hair was developed using SPE and GC/MS (*692*). The concentrations of khat constituents in blood and urine samples from subjects driving under the influence have been reported (*693*). Ephedra alkaloids were determined in urine and plasma by HPLC−UV (*694*).

Tramadol was determined in hair by SPE and GC/MS (*695*). A procedure was developed for the determination of trimeperidine and ketamine in whole blood using GC/MS (*696*). Ketamine was determined in urine by LC/MS/MS (*697*), GC/isotope dilution MS (*698*), and GC/MS (*699*) and in the blood by GC/NPD (*700*).

LC/MS/MS was used to detect zolpidem in hair (*701*) and in oral fluid (*702*) and zopiclone in hair (*703*). A HPLC method with fluorescence detection for the determination of fenfluramine and norfenfluramine in human hair has been described (*704*). Molindone was identified by GC/MS and quantitated using GC/NPD (*705*). Clomipramine and norclomipramine were identified and quantitated in tissues recovered from two postmortem cases using LC/MS (*706*). Sertraline was detected in the blood by SPE and GC/MS (*707*). Buprenorphine was detected by ELISA and confirmed by HPLC/MS (*708*). Colchicine was determined in biological specimens by HPLC-DAD (*709*). Fenarimol was determined in biological specimens by HPLC/DAD/MS (*710*). Stychnine has been determined in biological specimens by LC/DAD (*711*), and HPLC (*712*).

Analytical techniques for cyanide in blood and published blood cyanide concentrations from healthy subjects and fire victims have been reviewed (*713*). Cyanide and mercury were identified and quantified using HPLC/DAD in fluorescence mode and ICP with mass selective detector (ICPMS) (*714*). Pholedrine was detected in biological samples from a fatal intoxication case by LC/MS/MS (*715*). Xenobiotics were analyzed in biological fluids by ^{1}H NMR (*716*). Stability and precision studies were conducted for the chemical analytes in vitreous humor (*717*). A sensitive and simple method for the simultaneous determination of formate and acetate in whole blood and urine using GC/MS has been described (*718*). Identification of glyphosate in biological fluids from cases of poisoning has been performed by using a combination of ^{1}H and ^{31}P NMR (*719*).

A simple and rapid procedure for the determination of methylparathion in postmortem biological samples was developed using HS-SPME and GC/NPD (*720*).

Other Techniques. Reviews on the forensic analysis of drugs have been published (*721*, *722*). Drugs and other substances of forensic interest have been analyzed by GC/MS (*723*−*727*), LC/MS/MS (*728*−*740*), CE (*741*−*744*), Raman spectroscopy (*745*−*748*), HPLC (*749*−*753*), HPLC-ESI-MS (*754*), LC/TOFMS and LC/chemiluminescence nitrogen detector (*755*), GC × GC/TOFMS (*756*), GC with retention time locking (*757*), GC/MS and GC/ECD (*758*), hydrophilic interaction chromatography−mass spectrometry (*759*), SPME and GC/MS (*760*−*764*), immunoassays (*765*−*770*), LC-ES-MS (*771*), overpressured-layer chromatography and GC/MS (*772*), GC/MS/MS (*773*), LC/TOFMS (*774*), HS-SPME and GC (*775*), NMR (*776*−*779*), SPE and LC/MS/MS (*780*), TLC (*781*), and SPME and LC/MS (*782*).

New software was developed to simplify the total ion current chromatogram in order to improve the chances for identification of a "general toxicology unknown" (*783*). A field evaluation of five on-site drug-testing devices has been reported (*784*).

Examinations of proficiency test samples in the analysis of hair for drugs of abuse were performed by immunoassay, GC/MS, GC/MS/MS, and LC/MS/MS (*785*). The concentrations of two new standard reference materials for the determination of drugs of abuse in human hair were performed by GC/MS and LC/MS (*786*). Recent improvements in forensic hair analysis for illicit drugs have been reviewed (*787*).

An evaluation of rapid point-of-collection oral fluid drug-testing devices has been reported (*788*). The gas chromatographic Kovats retention indices of relevant forensic toxicologically compounds have been predicted (*789*).

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