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Forensic Science

T. A. Brettell*

Forensic Science Laboratory Bureau, New Jersey State Police, Box 7068, West Trenton, New Jersey 08628-0068

N. Rudin

1563 Solano Avenue, PMB506, Berkeley, California 94707-2116

R. Saferstein

Box 1334, Mount Laurel, New Jersey 08054

Review Contents

It is the aim of this review to present a concise survey of articles appearing in publications that primarily appeal to forensic practitioners. To accomplish this objective, we have focused our attention on the following journals: *Journal of Forensic Sciences*, *Science & Justice*, *Forensic Science International*, *Journal of the Canadian Society of Forensic Science*, *Forensic Science Review*, *Journal of Analytical Toxicology*, *Electrophoresis*, and *BioTechniques*, as well as *Chemical Abstracts Selects: Forensic Chemistry*. Our survey encompasses the period from January 2001 through December 2002. 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

Since the last *CA Selects* review article published in 2001, numerous studies addressing the development and validation of short tandem repeat (STR) DNA typing systems have been published. This has answered criticisms regarding the dearth of substantive peer-reviewed publications in this area. The method is now well-accepted as the standard in forensic DNA analysis and is rarely challenged in court. Numerous population genetic studies have also been performed using the various STR systems. Because of space limitations, we include only a few large population studies for autosomal STRs at the end of this section.

The literature addressing Y-chromosomal STRs (Y-STRs) and mitochondrial DNA (mtDNA) continues to accumulate. As competing commercial kits become available, Y-STR testing is likely to continue to gain favor with the forensic community. Several papers addressing the limitations of Y-STR population genetics are listed. While a commercial kit for mtDNA sequencing has not yet been offered, a kit for mtDNA testing using immobilized probes is currently under development. A paper describing this system is listed in this section. As for autosomal STRs, numerous population studies have been performed for Y-STR and mtDNA typing systems. Again, space considerations limit us to cite the largest and most general such studies.

Several methods for the extraction of DNA from difficult samples are presented as well as some new developments in the identification of body fluids. It is not clear yet whether these will be adopted by the community. The lack of a reliable and efficient DNA quantification method has been an ongoing complaint of the forensic DNA community. Several alternatives are presented. More sensitive DNA analysis techniques mean not only that minute crime scene samples can be typed but that unusual items can serve as secondary reference samples. A couple of papers address this issue. Along the same line of getting more from less,

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a method is proffered for the typing of genomic DNA recovered from a PCR reaction Finally, several studies address the issue of transfer. This defines a critical junction in forensic DNA typing. As the attribution of evidential source is rarely questioned anymore, the challenges have shifted to the question of contact—how did the DNA get there? This trend is expected to continue.

Another area in which rapid development has taken place is the use of DNA in forensic entomology. DNA can be typed both from insect larvae and from human tissue they might have ingested. These techniques are becoming important tools in forensic investigation. A paper on soil analysis using DNA and, of current interest, a paper discussing a solid-state chip for identifying pathogens are also included.

A new section for this review is DNA databases. As the use of convicted felon databases grows, the community has been presented with both technical and legal challenges. Several papers discussing these issues are listed. The statistical estimation of the strength of a DNA match continues to generate discussion, at least at the academic level, and these papers are included as well. Finally, several general reviews of DNA typing are listed.

Detection, Preservation, Extraction, and Quantitation of Biological Material. A method is presented to identify and quantitate contaminating DNA present in chemistry, on equipment, or introduced by operator handling (*1*), Unusual sources such as urine, perspiration, razor blades, chewing gum, ear wax, toothbrushes, envelope flaps, postage stamps, fingernails, and wrist watches have been successfully used as secondary standards in PCR-based analysis (*2*). The development of an enzyme-linked immunosorbent assays (ELISA) for Gm (1) and Gm (3) markers in blood, semen, and vaginal secretions is described (*3*). Results indicate that a silica-based extraction method produces mtDNA suitable for genetic identification from hair shafts and teeth (*4*). DNA extracted from liquid blood samples stored at 4 °C for 15 years was successfully typed (*5*). Three DNA extraction procedures, standard organic extraction, QIAamp spin columns, and Dynabeads, were compared for paraffin-preserved tissue samples and stained microscope slides (*6*). The use of DNA extracted from FTA cards in small-volume PCR reactions was investigated (*7*). The use of a CCD camera imaging system as a recording device to quantify human DNA by slot blot hybridization was investigated (*8*). The stability of seven STR loci, VWA31/A, TH01, F13A1, FES/FPS, APOAI1, D11S554, and ACTBP2, in human postmortem tissues was studied (*9*). It was discovered that the use of luminol to test for the presence of blood traces is more specific when the peak emission wavelength of the luminol chemiluminesence is measured (*10*). It was discovered that a number of substances (parsnip, turnip, horseradish, and enamel paint and, to a lesser extent, terracotta or ceramic tiles, polyurethane varnishes, and jute or sisal matting) could be mistaken for blood when detected with luminol (*11*). A dot-blot immunoassay for semen identification was developed using a polyclonal antibody against semenogelin, a seminal marker (*12*). It was demonstrated that lip cosmetics can be a good source for reference DNA (*13*). It was shown that human serum contains fragmented DNA derived from apoptosis of leukocytes that is useful for DNA typing (*14*). The "OneStep ABAcard PSA test" kit was validated for casework (*15*). It was demonstrated that GenoFix, a new tissue preservation solution for mass disaster DNA identification, effectively preserves tissue and DNA, making it useful in collecting samples from remote disaster locations (*16*). Extraction of human DNA for PCR from chewed residues of betel quid is most efficient using a novel poly(vinylpyrrolidone) and cetyltrimethylammonium bromide method, which binds two common classes of PCR inhibitors in plants, polyphenols, and polysaccharides (*17*). Nucleic acid isolation and purification from complex matrixes such as food was investigated (*18*). Three kits for the detection of prostate specific antigen (PSA) in semen were compared for sensitivity and specificity (*19*). The influence of cyanoacrylate on the efficiency of PCR was investigated (*20*). The use of shed epithelial cells recovered from bed sheets as secondary standards is reported (*21*). Four different filter papers were compared for the quantitative recovery of DNA from aged blood stains (*22*). The use of DNA analysis to assist in the interpretation of bloodstain patterns is explored (*23*). A procedure for typing DNA from unbuffered formalin-fixed paraffin-embedded tissues is described (*24*). Variables affecting the recovery of DNA from common drinking containers are compared (*25*). The mDNA and STR typing of matter adhering to an earphone is described (*26*). A sensitive method for the quantitation of genomic DNA by Alu amplification is presented (*27*). The effect of 1,2-indanedione, a latent fingerprint detection reagent, on subsequent DNA profiling is described (*28*). The theory and application of the transfer of trace quantities of DNA through skin contact is reviewed (*29*). A procedure for real-time DNA quantification of nuclear and mtDNA in forensic analysis is described (*30*). A method for recovering genomic DNA from archived PCR product mixes for subsequent multiplex amplification and typing of additional loci is described (*31*). The propensity of individuals to deposit DNA and secondary transfer of low-level DNA from individuals to inert surfaces is explored (*32*). Extraction of human nuclear DNA from feces samples using the QIAamp DNA Stool Mini Kit is reported (*33*). The identification of menstrual blood using RNA markers was evaluated (*34*).

Short Tandem Repeats (STRs). An eroded connector for the cathode mounted on the heat plate assembly was identified as the cause of a migration anomaly on an ABI Prism 310 Genetic Analyzer. (*35*) A new seven-STR multiplex system (third-generation multiplex) is described (*36*). A genotyping inconsistency at the STR vWA between the AmpF/STR Profiler Plus kit and the GenePrint CTTv kit is reported (*37*). Full profiles using the nine-locus AmpF/STR Profiler Plus multiplex system were obtained from known and questioned DNA samples that had been digested by the *Hae*III restriction enzyme and stored at −70 °C for up to 8 years; however, in the six-locus AmpF/STR COfiler systems TH01, TPOX, and CSF1PO failed to amplify (*38*). The use of high-performance liquid chromatography (HPLC) and time-of-flight mass spectrometry (TOF-MS) methods for characterization of commercially available STR kits is presented (*39*). A large number of alleles at six STR loci, FGA, D3S1358, vWA, CSF1PO, TPOX. and TH01, were sequenced for both humans and higher primates (*40*). STR typing by capillary electrophoresis was validated for samples amplified using AmpF/STR Profiler Plus and COfiler kits and detected on the ABI Prism 310 Genetic Analyzer (*41*). STR typing for forensic usage was validated for samples amplified using AmpF/STR Profiler Plus and COfiler kits and detected on the ABI Prism 310 Genetic Analyzer, the ABI Prism 377 DNA Sequencer, and the FMBIO II Fluorescent Imaging Device (FluorImager)

(*42*). The AmpF/STR Profiler Plus PCR Amplification Kit was validated for use in forensic casework (*43*). A concordance study comparing population database samples typed using the PowerPlex 16 kit and the AmpF/STR Profiler Plus and COfiler Kits for 500 population database samples comprising African Americans, Bahamians, and Southwestern Hispanics produced only one sample in which a typing difference was observed (*44*). A phenotypic difference, likely due to a primer binding site mutation, at the vWA locus amplified with different STR typing kits is described (*45*). The use of STRs in forensic DNA typing is reviewed (*46*). The AmpF/STR Profiler Plus and AmpF/STR COfiler STR Multiplex Systems using capillary electrophoresis for separation were validated for forensic DNA typing (*47*). The characteristics of profiles produced with the AmpF/STR SGM Plus multiplex system for both standard and low copy number STR DNA analysis were compared (*48*). STRBase, an on-line STR DNA database for the human identity testing community is introduced (*49*). The sizing of STR alleles in capillary array gel electrophoresis instruments is discussed (*50*). Allele distributions for 12 or 13 CODIS core tetrameric STR loci (CSF1PO, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, FGA, TH01, TPOX, vWA) were detected in 41 population data sets comprising African Americans, U.S. Caucasians, Hispanics, Far East Asians, and Native Americans (*51*). The results from interlaboratory comparison of DNA quantification practice and STR multiplex performance with multiple-source samples are presented (*52*). Allele frequencies for 14 STR loci of the PowerPlex 1.1 and 2.1 multiplex systems and the penta D locus in Caucasians, African-Americans, Hispanics, and other populations of the United States and Brazil were determined (*53*). Past, present, and future perspectives for the role of STRs in forensic casework in the U.K. are reviewed (*54*). The TWGDAM validation of AmpF/STR PCR amplification kits for forensic DNA casework is presented (*55*). The process of constructing universal multiplex PCR−STR systems for comparative genotyping is described (*56*). Detection of a primer binding site polymorphism for the STR locus D16S539 using the Powerplex 1.1 system and validation of a degenerate primer to correct for the polymorphism is presented (*57*). An instance of somatic mutation at an STR locus resulting in a three-allele pattern and mosaicism is described (*58*). PowerPlex 16, a commercial STR kit comprising a 16-locus fluorescent multiplex system was validated for forensic use (*59*). Characterization and validation studies of PowerPlex (*60*) 2.1, a nine-locus STR multiplex system and penta D monoplex are presented (*60*). The use of reduced-volume PCR amplification reactions with the AmpF/STR Profiler Plus Kit was investigated (*61*).

Y-STR Typing and Gender Identification. Combining DYS385 with other fluorescently labeled Y-STR primers in a multiplex reaction resulted in detection of polymorphic female amplification products for DYS391, which is also situated on the human X-chromosome (*62*). Male-specific polymorphisms used in forensic DNA typing are reviewed (*63*). Mutation rates and mechanisms for two human Y-STR loci, DYS19 and DYS390, were characterized using small-pool PCR techniques (*64*). Samples exhibiting abnormalities were identified at amelogenin and several Y-STR loci used in forensic testing (*65*). The genes and biological functions of the human Y chromosome and its use in investigating the origin and history of human populations are reviewed (*66*). The use of the LightCycler for detection of Y chromosome SNPs is discussed (*67*). Y-Chromosomal SNP haplotype diversity is investigated (*68*). Y-STR mutations detected in forensic cases are presented (*69*). Routine Y-STR typing in forensic casework is described (*70*). The application of Y-STR analysis in a rape case is presented (*71*). Application of a Y-STR pentaplex (DYS19, DYS389I and II, DYS390 and DYS393) in sexual assault cases is discussed (*72*). The implication of mutations at Y-STR loci for paternity testing and forensic analysis is discussed (*73*). The results of a collaborative study of the EDNAP group regarding the reproducibility and robustness of the Y-chromosome pentaplex DYS19, DYS389 I and II, DYS390 and DYS393 is presented (*74*). A method for sex assignment in mixed samples by amplifying a specific amelogenin Y sequence is described (*75*). Validation and casework application of a Y-STR multiplex is presented (*76*). The sequence structure of 12 novel Y-STRs and PCR amplification strategies are discussed (*77*). Six new Y-specific STR markers (DYS434, DYS435, DYS436, DYS437, DYS438, DYS439) are described and named (*78*). A large reference database, of highly informative Y-chromosomal STR haplotypes, representing the largest collection of male-specific genetic profiles currently available for European populations, is available on-line at http://ystr.charite.de (*79*). The DNA commission of the International Society of Forensic Genetics provides recommendations on forensic analysis using Y-STRs (*80*). Tests based on detection of X- and Y-chromosome-specific sequences within the amelogenin gene, as developed for forensic samples, have been applied to ancient DNA with varying degrees of success (*81*). A procedure for typing recalcitrant Y-STRs using redesigned primers is presented (*82*). Foreign DNA extracted from the fingernail clippings of female victims may be characterized using PCR−Concert rapid purification and by typing the DYS19 locus (*83*). A nomenclature system for the tree of human Y-chromosomal binary haplogroups is presented (*84*). The efficacy of Y-STR typing in sexually assaulted female victims with no cytological detection of spermatozoa is described (*85*). Nine Y-STRs were analyzed using the quadruplex (DYS393, DYS19, DYS392, DYS385−I/II) and a triplex PCR (DYS390, DYS389−I/II, and DYS391) (*86*). The use of chemical composition of fingerprints for gender determination is investigated (*87*). A new pentaplex system to study short tandem repeat markers of forensic interest on X chromosome is described (*88*), A multiplex PCR assay capable of simultaneously amplifying 20 Y-STRs (the "extended haplotype" used in Europe: DYS19, DYS385, DYS389I/II, DYS390, DYS391, DYS392, DYS393, and YCAII and DYS437, DYS438, DYS439, DYS447, DYS448, DYS388, DYS426, GATA A7.1, and GATA H4) has been developed (*89*). Fourteen novel Y-STRs (eight tetranucleotide repeats; DYS449, DYS453, DYS454, DYS455, DYS456, DYS458, DYS459, and DYS464, five pentanucleotide repeats, DYS446, DYS447, DYS450, DYS452, and DYS463, and one hexanucleotide repeat,DYS448) were identified, characterized. and typed for a globally diverse panel of 73 cell lines and 148 individuals from a European-American population (*90*).

Mitochondrial DNA Typing. Mitochondrial DNA typing is reviewed (*91*). The detection of sequence variation in the HVII region of the human mitochondrial genome using immobilized sequence-specific oligonucleotide probes is described (*92*). Controversies over heteroplasmy in mtDNA are discussed (*93*). A pool of European mtDNA samples have been analyzed for recombina-

tion (*94*). A rapid and convenient method for quantitating human mitochondrial DNA using a real-time PCR is described (*95*). An improved mtDNA sequence analysis of forensic remains using a "miniprimer set" amplification strategy is presented (*96*). The mtDNA substitution rate and segregation of heteroplasmy were studied for the noncoding control region (D-loop) and 500 bp of the coding region in 194 individuals representing 33 maternal lineages (*97*). The use of mtDNA in anthropology, evolutionary research, and forensic testing is reviewed (*98*). It was demonstrated that, for different hairs within an individual, the HV2 C-stretch region can vary with respect to the number of cytosines and/or proportion of C-stretch length variants (*99*). Human hair histogenesis for the mtDNA forensic scientist is reviewed (*100*). Comparative mtDNA analysis of the putative heart of Louis XVII with the remains of Carl Wilhelm Naundorff gave evidence that his remains could not be identified as those of Louis XVII (*101*). A protocol for mtDNA sequence analysis for forensic identification using pyrosequencing technology is presented (*102*). Fluorescent single-strand conformation polymorphism of overlapping fragments has been developed as a highly sensitive method for the screening of mtDNA variation (*103*). Considerations by the European DNA profiling (EDNAP) group on the working practices, nomenclature, and interpretation of mDNA profiles are presented (*104*). Results of the 1999−2000 collaborative exercise and proficiency testing program on mtDNA of the GEP−ISFG on the observed variability in the heteroplasmy level of hair from the same donor are presented (*105*). A procedure is presented for the quantification of human mtDNA in a real-time PCR (*106*). A critique of Grzybowski provides alternative explanations for the apparent high levels of heteroplasmy observed in the human mtDNA hypervariable region I from hair in that study (*107*). An improved method for post-PCR purification for mtDNA sequence analysis is described (*108*). Recommendations for the consistent treatment of length variants in the human mtDNA control region are suggested (*109*). A method to obtain reliable mtDNA sequences downstream of homopolymeric stretches with length heteroplasmy in the sequencing direction is described (*110*). Successful DNA typing of a urine sample in a doping control case using human mtDNA analysis is described (*111*). Caucasian haplogroups present in the SWGDAM forensic mtDNA data set for 1771 human control region sequences were characterized (*112*).

Other DNA Typing Systems. The utility of minisatellite variant repeat mapping by PCR in a paternity case showing a false exclusion due to an STR mutation is presented (*113*). The utility of single-nucleotide polymorphisms (SNPs) for forensic use is assessed (*114*). The detection of double-stranded PCR amplicons at the attomole level electrosprayed from low-nanomolar solutions using FT-ICR mass spectrometry is described (*115*). The application of capillary electrophoresis to forensic DNA typing is reviewed (*116*). The use of DNA profiling to infer the ethnic origin of a crime stain is reported (*117*). The use of single-cell gel electrophoresis, also known as the comet assay, to evaluate postmortem cell death processes, specifically nuclear DNA fragmentation, is assessed (*118*).

Forensic Entomology and Other Nonhuman DNA Typing Systems. The typing of both human and insect mtDNA from maggots is described (*119*). The forensic application of allozyme electrophoresis to the identification of blowfly larvae (*Diptera: Calliphoridae*) in southern Australia is discussed (*120*). The genetic relationships between blowflies (*Calliphoridae*) of forensic importance are described (*121*). A method for using mtDNA sequence data and phylogenetic analysis to identify specimens of the blow fly subfamily *Chrysomyinae* likely to be collected from a human corpse within Canada or the United States is described (*122*). P30 (PSA) results and Y-STR profiles were obtained from larvae infesting a cadaver (*123*). The use of beetles in forensic entomology is discussed (*124*). The history of forensic entomology is reviewed (*125*). The DNA-based identification and molecular systematics of *Sarcophagidae* (*Diptera*) are presented (*126*). A method for surface sterilization of maggots using bleach that does not interfere with mtDNA analysis of crop contents is described (*127*). Results from mtDNA sequencing of beetle larvae (*Nitidulidae: Omosita*) recovered from human bone are presented (*128*). The forensic comparison of soils by bacterial community DNA profiling is presented (*129*). The sequence-specific identification of 18 pathogenic microorganisms using microarray technology is described (*130*).

DNA Databases. A simple and reliable strategy for high-throughput processing of reference samples for DNA intelligence databases is described (*131*). Criminal DNA databases in Europe are discussed (*132*). A brief history of the formation of forensic DNA databases within Europe is presented (*133*). Regulations regarding the collection and use of DNA information are set forth (*134*). The use of the DNA database to solve identity fraud problems is discussed (*135*).

Interpretation and Statistical Weight of DNA Typing Results. A two-locus match probability is presented that incorporates the effects of within-subpopulation inbreeding (consanguinity) in addition to population subdivision (*136*). Eleven populations across Europe were analyzed, and the estimate for q over all 13 CODIS core STR loci, 0.0028, was found to be much lower than the q estimate of 0.01 advocated by the second National Research Council Report in 1996 (*137*). Bayesian posterior distributions are obtained for the time to the most recent common ancestor for a nonrecombining segment of DNA (such as the nonpseudoautosomal arm of the Y chromosome or the mitochondrial genome) for two individuals given that they match at k out of n scored markers (*138*). Forensic evaluation of Y-STR haplotype matches is discussed (*139*). A Bayesian approach to analyzing multilocus genotype or haplotype data to assess departures from gametic (linkage) equilibrium is described (*140*). The evaluation of the DNA match probability in criminal cases is discussed (*141*). An exact test for Hardy−Weinberg equilibrium in the biallelic case, based on the ratio of weighted likelihoods under the null and alternative hypotheses, is presented (*142*). Linear mixture analysis, a mathematical approach to resolving mixed DNA samples, is described (*143*). The current state of the practice with regard to the application of the principles of population genetics to the interpretation of DNA profiling evidence is discussed (*144*). Mismatch distribution analysis of Y-STR haplotypes is presented as a tool for the evaluation of identity-by-state proportions and the strength of matches (*145*). A statistical procedure for bounding the number of contributors to mixed DNA stains is discussed (*146*).

General Reviews. DNA standard reference materials developed by the National Institute of Standards and Technology are reviewed (*147*). Quality assurance in forensic DNA typing is discussed (*148*).

TRACE EVIDENCE

Petroleum Products and Explosives. A number of solvents have been evaluated for the ability to desorb accelerants from charcoal strips (*149*). Forensic analysis of ignitable liquids in fire debris by comprehensive two-dimensional gas chromatography has been reported (*150*). Detection of gasoline in fire debris has been reported by gas chromatography/mass spectrometry/mass spectrometry (GC/MS/MS) (*151*). Purge-and-trap GC/MS for forensic examination of oil stains was studied (*152*). Forensic analysis of commercial petroleum products using laser-induced fluorescence detection has been reported (*153*). The persistence of floor coating solvents has been examined (*154*). Degradation of gasoline, barbecue starter fluid, and diesel fuel by microbial action in soil was studied (*155*). Arson and incendiary fires, chemistry of fire, determination of cause and origin, evidence collection and storage, nature of potential accelerants, and current practices of forensic laboratories has been reviewed (*156*). FTIR has been demonstrated as a useful analytical tool for differentiating motor oils (*157*). A comparative analysis of lubricants used for weapons has been reported (*158*).

Detection limits for GC/MS analysis of organic explosives have been reported (*159*). Forensic identification of explosive oxidizers by electrospray ionization mass spectrometry has been reported (*160*). A rapid and simple field test for the detection of triacetone triperoxide and hexamethylene triperoxidediamine, two explosives that find significant illegal use, has been developed (*161*). The detection and identification of traces of inorganic ions and sugars can play a major role in the forensic investigation of an explosives-related incident. The background levels of these substances in the general environment have been investigated (*162*). Methylene blue color test reagents used for the detection of the perchlorate anion have been modified for use as a microchemical test for perchlorates and chlorates (*163*). Capillary electrophoresis has been used for the analysis of inorganic ions commonly found in postblast explosive residues (*164*).

GC/MS analysis of explosives has been reviewed (*165*) The analysis of explosives has been reviewed (*166*). The forensic analysis of explosives by LC/MS has been reviewed (*167*). Field detection and monitoring of explosives has been reviewed (*168*). The application of ion mobility spectrometry for the detection of explosives and explosive-related compounds has been reviewed (*169*). The viability of hair as a surface from which explosive traces can be recovered has been studied and it showed that as little as 1-h vapor exposure can result in measurable traces of explosives (*170*). The use of dogs as chemical detectors and the scientific foundation and available information on the reliability of explosive detector dogs has been reviewed(*171*). Pyrotechnic residues were analyzed by scanning electron microscopy/energy-dispersive spectroscopy (*172*).

Hair, Fibers, and Paint. Correlation of microscopic and mitochondrial DNA hair comparisons was reported (*173*). Macroscopic and microscopic hair specimen classifications are provided as a guide to associate mitochondrial DNA analysis with the physical characteristics of hair (*174*). No correlation of time of death with scalp hair proximal end decomposition has been found (*175*).

Single fibers dyed by indigo and its derivatives were measured by microspectrophotometric examination (*176*). Raman spectra of single fibers were obtained directly from glass slide mounts (*177*). The recovery of fibers from fingernail clippings has been evaluated (*178*). Black cotton fibers were shown to have a high degree of diversity in their spectral patterns (*179*). Lyorell fibers were characterized using dispersion staining (*180*). The forensic characterization of black cellulose fibers has been evaluated (*181*). Raman microspectroscopy has been found to be applicable to the forensic analysis of colored fibers (*182*). Single-fiber analysis by internal reflection IR has been described (*183*). A new type of polyester fiber, polytrimethylene terephthalate, has been evaluated (*184*). Single textile fibers were analyzed by sample-induced isotachophoresis and micellar electrokinetic capillary chromatography (*185*). The forensic value of fibers has been reviewed (*186*). Crime laboratories have been surveyed with respect to fiber examination techniques (*187*).

A study was undertaken to evaluate the discrimination power of common forensic techniques when applied to the newer generation original automotive finishes. The results demonstrate that IR spectroscopy is an effective tool for discriminating between the major automotive paint manufacturers' formulation types that are currently used in original finishes (*188*). Laser micropyrolysis gas chromatography/mass spectrometry has been used for the forensic analysis of paint, photocopier toner, and fibers (*189*). Raman spectroscopy was used to rapidly screen automotive paints for certain paint pigments (*190*).

Gunshot Residues and Bullet Analysis. It was found that there is no substantial danger of not detecting gunshot residue (GSR) by a tape lift method (*191*). GSR particles from hands, hair, face, and clothing were characterized using SEM/wavelength-dispersive X-ray analysis (*192*). Distribution of lead and barium in gunshot residue particles derived from 0.22 caliber rimfire ammunition has been examined (*193*). GSR originating from six types of pistol ammunition were studied. The study revealed some differences in the frequencies of occurrence of certain classes of primer residues (*194*). GSR residue analysis has been reviewed (*195*). Proficiency test results were reported on the identification of gunshot residue by scanning electron microscopy/energy-dispersive X-ray (*196*). The application of time-of-flight secondary ion mass spectrometry as a complementary technique to SEM-EDX for the characterization of GSR from 0.22 caliber rimfire ammunition has been reported (*197*).

Indicators of the type of weapon and ammunition used can be obtained from the distribution of GSR particle shapes and from the multielement analysis of the firearms discharge residue sample (*198*). Micellar electrokinetic capillary electrophoresis has been found to be a potentially valuable tool in the examination of GSR evidence for characteristic organic gunpowder compounds (*199*, *200*). The effect of changing ammunition on the composition of organic additives in gunshot residue has been investigated (*201*). A study associating gunpowder and residues from commercial ammunition using compositional analysis was reported (*202*). Brake linings have been shown to be a source of non-GSR particles containing lead, barium, and antimony (*203*). An HPLC procedure

for analyzing smokeless powder has been reported (*204*). Extraction technique for determining the organic additives in smokeless handgun powder have been studied (*205*).

Detection of firearm imprints on the hands of suspects using pyridyldiphenyl triazine has been reported (*206*). Bullets were characterized by lead isotope ratio and trace element analysis (*207*). A metallurgical review of the interpretation of bullet lead compositional analysis has been undertaken (*208*). Trace elements in lead alloys produced by two smelters and one ammunition manufacturer were determined (*209*). A capillary electrophoretic method for nitrite and nitrate determination as a screening tool for investigating the residues of firearm discharge has been described (*210*).

Fingerprints. Determination of the chemical nature of fingerprints to ascertain whether differences in chemical markers can be used to determine personal traits, such as age, gender, and personal habits, has been udertaken (*211*). Enhancement of the ninhydrin reaction with latent fingerprints using a suitable post-treatment process has been reported (*212*). The presence of ninhydrin on a bloodstain can lead to the failure of the Leuco Crystal Violet test for blood (*213*). 5-Alkoxyninhydrins were examined as latent fingerprint visualization reagents (*214*). Six ninhydrin analogues were evaluated as fingerprint development reagents (*215*). Excellent performance of 1,2-indanedione as a latent fingerprint detection reagent has been reported (*216*). The effect of 1,2-indanedione, a latent fingerprint reagent, on subsequent DNA profiling has been studied (*217*). Chemical processes involved in the development of latent fingerprints using the cyanoacrylate fuming method have been studied (*218*). Silver physical developers for the visualization of latent prints on paper has been reviewed (*219*). The presence of cyanoacrylate significantly hampered the amplification of DNA from small stains whereas there was virtually no difference comparing the amplification results of DNA extracted from bigger stains (*220*). Powder methods for the detection of latent fingerprints were reviewed (*221*). The utilization of the lanthanide shift reagent tris (6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium(III) [Eu(fod)$_3$] as a simple one-step reagent for the luminescent visualization of latent fingerprints has been investigated (*222*).

Miscellaneous. Ballpoint inks have been characterized by electrospray ionization mass spectrometry (*223*). HPLC analysis of ballpoint pen inks stored at different light conditions has been reported (*224*). Bulk blue ballpoint pen ink has been classified by FT-IR (*225*). A method for comparison of the relative age of ink entries written by the same ballpoint pen on documents stored in darkness has been presented (*226*). Desorption/ionization mass spectrometric methods in the forensic applications of the analysis of inks on paper have been examined (*227*). Ballpoint pen inks were analyzed by HPLC (*228*).

The differentiation of float glass using refractive index and elemental analysis has been evaluated (*229*). The concentrations of 10 elements in unrelated glass specimens received as evidence were used to assess the frequencies of errors of false association (*230*). The FBI has selected nine elements to use for discrimination among the glass samples. This multielement discrimination has been shown to be a significant improvement in the discrimination statistics over using only refractive index measurements (*231*). The application of capillary electrophoresis to forensic soil analysis has been examined (*232*). An investigation has shown that a soil microbial community DNA profile can be obtained from the small sample of soil recovered from the sole of a shoe and from soil stains on clothing (*233*). Black toners were characterized using FT-IR and pyrolysis-GC/MS (*234*). A methodology based on NMR spectroscopy was reported for the forensic analysis of condoms (*235*). A protocol for the forensic analysis of condom and personal lubricants found in sexual assault cases has been reported (*236*). Various applications of capillary electrophoresis in the analysis of forensic specimens were reviewed (*237*).

DRUGS AND POISONS

Ethanol and Volatiles. The Alcotest 7100 MKIII Dual C breath alcohol analyzer was evaluated according to the Canadian Society of Forensic Sciences Alcohol Test Committee standards for approved instruments (*238*). The effect of swallowing or rinsing alcohol solution on the mouth alcohol and slope detection of the Intoxilyzer 5000 has been studied (*239*). A study of the simulation of the effect of blood in the mouth on breath alcohol concentrations of drinking subjects concluded that blood in the mouth does not lead to an overestimation of the breath alcohol concentration of drinking subjects (*240*). Methyl *tert*-butyl ether was evaluated as interference on two breath alcohol analyzers (*241*). The storage stability of an aqueous alcohol simulator solution stored in polyethylene bottles was studied (*242*). A method that uses compressed gas ethanol breath standard cylinders to perform periodic determination of accuracy on evidential breath alcohol instruments has been developed (*243*). The applicability of the quantitative ethanol detector (QED) test kit for screening of ethanol concentrations in blood samples was investigated (*244*). The QED test kit was evaluated for the determination of postmortem ethanol levels (*245*). The automated headspace solid-phase microextraction (SPME) and capillary GC analysis of ethanol in postmortem specimens has been described (*246*). Ethanol was determined in blood and urine by headspace GC to study the reference limits for urine/blood ratios of ethanol in two successive voids from drinking drivers (*247*). A method of analyzing seized/suspected beverages for their alcohol content using the Intoxilyzer 5000C or the Alcotest 7410 GLC has been reported (*248*).

The determination of ethanol and other volatile organic compounds in biological fluids has been described using headspace GC (*249*, *250*) and headspace SPME (*251*, *252*). Cyanide in blood was determined by HPLC (*253*), headspace GC/MS (*254*), and capillary electrophoresis with fluorescence detection (*255*).

Cannabinoids. A comparison of Roche kinetic interaction of microparticles in solution assay for cannabinoids and GC/MS analysis for 11-nor-9-carboxy-Δ-trahydrocannabionol (THCCOOH) has been reported (*256*). The effects of oxidizing adulterants on detection of 11-nor-Δ-9 THC-carboxylic acid in urine specimens has been studied (*257*). Temporal indication of marijuana use can be estimated from plasma and urine concentrations of cannabinoids (*258*). Cannabinoids have been determined in biological fluids by GC/MS (*259*−*261*), GC/negative ion chemical ionization-mass spectrometry (NICIMS) (*262*), and LC/MS/MS using negative atmospheric-pressure chemical ionization (APCI) (*263*). A screening technique for the simultaneous cleanup, extraction,

and derivatization of 11-nor-Δ-9-THCCOOH from urine has been developed using hollow fiber membrane solvent microextraction (HFMSME) with in-tube derivatization and GC/MS (*264*). A GC/MS confirmation method has been described using large-volume programmed-temperature vaporization injection, for the determination of cannabinoids in blood samples with deuterium-labeled internal standards (*265*). Δ-9-THC, 11-OH-Δ-9-THC, and Δ-9-THCCOOH plasma or serum to whole blood concentrations distribution ratios in blood samples taken from living and dead people has been studied (*266*).

Oral fluid testing has been compared to urine testing by enzyme immunoassay (EIA) and GC/MS for the use of marijuana (*267*). The detection of cannabinoids in saliva samples was examined by SPME and GC/MS (*268*). Cannabinoids were determined in hair samples using high-pH nonaqueous electrolytes and electrochemical detection (*269*), negative ion GC/MS (*270*), and GC/MS-electron impact and GC/MS-NCI (*271*).

Morphine and Related Narcotics. Heroin and related narcotics have been studied by principal component analysis and Raman spectroscopy (*272*) and high-performance thin-layer chromatography (HPTLC) (*273*). A capillary zone electrophoresis (CZE) method for the rapid determination of heroin, secondary products, and additives present in clandestine heroin samples has been described (*274*). Methods for chemical profiling of heroin samples have been reviewed (*275*). Heroin profiling has been accomplished using GC/MS (*276*), capillary electrochromatography and laser-induced fluorescence detection (*277*), and electrothermal atomic absorption and inductively coupled plasma-atomic emission spectrometry (*278*). GC/MS has been used to determine the pyrolysis products of diacetylmorphine and adulterants of street heroin samples (*279*). An acetylating method for fast synthesis of heroin from morphine in the presence of 4-(dimethylamino)pyridine as a catalyst has been described (*280*).

Opiates have been determined in biological fluids by GC/MS (*281*−*284*), immunoassay and GC/MS (*285*, *286*), immunoassay and isotope-dilution GC/MS (*287*, *288*), GC/NICIMS (*289*, *290*), HPLC with diode array detection (DAD) and fluorescence detection (*291*), automated solid-phase extraction (SPE) with LC/MS/MS (*292*), HPLC-electrospray ionization-triple quadrupole mass spectrometry (*293*, *294*), HPLC-atmospheric pressure CIMS (*295*), high-resolution electrospray ionization-IMS (*296*), and high-field asymmetric waveform IMS with mass spectrometric detection (*297*). A procedure for simultaneous quantitation of 6-monoacetylmorphine and acetylcodeine in hair was developed that used SPE and GC/MS in selected ion monitoring mode (*298*). SPE followed by GC/MS was used to determine opiates in hair and oral fluid (*299*), and immunoassay and GC/MS/MS was used to analyze opiates in oral fluid (*300*). Bone and bone marrow were analyzed by fluorescence polarization immunoassay (FPIA) and GC/FID (*301*). Opiates were determined in meconium by SPE and GC/MS (*302*). Morphine was determined in necrophagous insects remains by RIA and GC/MS (*303*).

GC/PICIMS has been used to determine methadone and its metabolites in urine (304). Methadone and its metabolites have been determined in hair by headspace SPME and GC/MS (*305*).

Cocaine. A comparison of the transmission and internal reflection infrared spectra of cocaine has been reported (*306*). Accelerated solvent extraction was developed for the rapid extraction of cocaine and benzoylecgonine from coca leaves (*307*). CE has been used to simulataneously analyze coca alkaloids and sugars in illicit cocaine (*308*). Headspace SPME and GC/MS have been used for the analysis of residual solvent trapped into illicit cocaine exhibits (*309*). An aptamer-based colorimetric probe for cocaine has been developed (*310*). The change in chemical structure of cocaine in the presence of hydrogen peroxide, a main component of hair dye and decolorant treatments, was studied using HPLC/MS (*311*).

Benzoylecgonine extraction from urine was explored using SPE columns and an automated sample processor (*312*). Urine analysis of laboratory personnel preparing cocaine-training aids for dogs was performed using GC/MS (*313*). Cocaine and its metabolites have been analyzed in biological fluids using liquid−liquid extraction and GC (*314*), GC/MS (*315*, *316*), LC/MS/MS (*317*), HPLC/MS (*318*, *319*), HPLC (*320*), LC/APIMS (*321*), SPE and GC/MS (*322*), GC/FID and HPLC/UV (*323*), HPTLC/UV (*324*), solvent microextraction and GC (*325*), and HFMSME/GC (*326*). The detection of cocaine and metabolites in oral fluids has been accomplished by EIA and GC/MS (*327*) and HFMSME and GC (*328*). An evaluation of external cocaine contamination and the risk of false positives in the testing of hair for drugs of abuse have been reported (*329*). Cocaine and its metabolites have been determined in hair samples using HPLC/MS (*330*), and supercritical fluid extraction/GC/MS (*331*). Benzoylecgonine detection in vitreous humor has been reported using EIA and GC/MS (*332*). GC/MS was used to detect cocaine and its metabolites in breast milk (*333*), blood, urine, and milk (*334*). Cocaine, its metabolites, and heroin and its metabolites have been detected in sweat patches using EIA (*335*).

Amphetamines. A review of illicit amphetamine-like drugs and new synthetic drugs reported in Europe and the United States has been reported (*336*). Analysis methods for amphetamine-related compounds have been reported using HPLC and CE (*337*), IMS (*338*), TLC (*339*), GC/FID (*340*), CE/DAD (*341*), GC, and proton and carbon NMR, FT-IR, and GC/MS (*342*, *343*). Methylenedioxylated amphetamines have been analyzed by HPLC/UV and HPLC/fluorescence detection (*344*), nonaqueous CE/fluorescence detection (*345*), and surface-enhanced Raman scattering spectroscopy (*346*). A method for simultaneous enantiomeric separation of methamphetamine-related compounds in a single run has been described using a simple CZE with β-cyclodextrin as chiral selector (*347*). The pyrolysis products of dimethylamphetamine have been studied by GC/MS, headspace GC/MS, and LC/ESI/MS (*348*). Methamphetamine and other related stimulant samples were profiled using headspace SPME/GC/MS (*349*, *350*), capillary GC (*351*), and GC/MS, GC/PIMS, and GC/MS/MS (*352*). The combined use of δ^2H, δ^{13}C, and δ^{15}N isotopic data along with ^{2}H NMR was used to study the synthetic pathways used in the preparation of MDA and N-substituted homologues (*353*).

A review on the forensic issues of amphetamines has been published (*354*). A review of the literature has been reported on the relevant psychomotor effects of 3,4-methylenedioxymethamphetamine (*355*). Amphetamine-related compounds have been analyzed in biological fluids using GC/MS (*356*), SPE and GC/MS (*357*, *358*), GC/PIMS (*359*), GC/FT-IR (*360*), solvent microextraction (SME) and GC/pulsed discharge helium ionization

detector (*361*), SPME and GC/ECD or GC/MS (*362*), SPME and HPLC/ESI-MS (*363*), headspace SPME and GC/MS (*364*−*367*), immunoassay, HPLC/DAD, and GC/MS (*368*), immunoassay and GC/MS (*369*), fluorometric detection with flow injection analysis and on-line liquid−liquid extraction (*370*), nonaqueous CE/fluorescence spectroscopy (*371*), chiral HPLC (*372*), ESI-IMS/MS (*373*), LC/ESI-MS (*374*), and liquid-phase microextraction/FIA/APCI/MS/MS (*375*).

Amphetamines and related compounds have been analyzed in hair by HPLC/MS/MS (*376*), HPLC/MS (*377*), EMIT and GC/MS (*378*), HPLC/UV and HPLC/fluorescence detection (*379*), headspace SPME and GC/MS (*380*), and headspace solid-phase dynamic extraction and GC/MS (*381*). MDMA and its main metabolite were determined in sweat by Drugwipe and the results correlated with urine and blood results determined by GC/MS (*382*).

Benzodiazepines. The use, abuse, and toxicity of Flunitrazepam have been reported (*383*). Flunitrazepam and its metabolites have been analyzed in biological fluids by HPLC/APCI-MS/MS (*384*) and immunoassay and GC/MS (*385*). Bromazepam has been detected in urine by EMIT, TLC, and GC/MS (*386*). Benzodiazepines have been detected in biological fluids by GC/TOF-MS (*387*), GC/ion trap MS (*388*), SPME and GC/MS (*389*), ESI-IMS-MS (*390*), LC-APCI-MS (*391*), HPLC/ESI-MS/MS (*392*), and HPLC/UV and electrochemical detection at the hanging mercury drop electrode (*393*).

Benzodiazepines have been found in hair by SPE and GC/NCIMS (*394*), ELISA (*395*), and HPLC/DAD (*396*). Flunitrazepam and its metabolite have been detected in oral fluid by SPE and GC/NCIMS (*397*).

***γ*-Hydroxybutyrate.** The forensic issues and implications of the chemical interconversion of *γ*-hydroxybutyrate (GHB) and *γ*-butyrolactone (GBL) in aqueous media have been discussed (*398*). GHB and related compounds have been analyzed by dual-mode ion trap mobility spectrometry (*399*) and micellar electrokinetic chromatography (*400*). GC/MS has been used to detect GHL in wine (*401*). The results of analysis of GHB in blood from subjects arrested for impaired driving (*402*) and fatalities (*403*) have been reported. The effect of storage temperature on endogenous GHB levels in urine has been reported (*404*). Endogenous GHB levels have been determined in antemortem urine and blood (*405*). The production of GHB has been shown to increase in postmortem liver with time after death (*406*). An improved procedure for the analysis of GHB and ethylene glycol in whole blood has been published (*407*). GHB has been determined in biological fluids by GC/MS (*408*), GC/FID and GC/MS (*409*), and SPME and GC/ion trap MS (*410*). A rapid colorimetric test for the detection of GHB in human urine has been described (*411*).

Miscellaneous. The importance of vacutainer selection in forensic toxicological analysis of drugs of abuse was studied using FPIA immunoassays and GC/MS (*412*). The analysis of urine for LSD has been done by micellar electrokinetic chromatography (*413*), immunoassays (*414*), HPLC with fluorescence detection (*415*), and LC/MS (*416*). The analysis of psilocyn and psilocybin in developmental mushrooms has been described (*417*). Psilocyn has been detected in human urine by GC/MS with derivatization with MSTFA (*418*). LC/MS was used to determine ketamine in urine (*419*). A survey of reported synthesis of methaqualone and some positional and structural isomers has been reported (*420*). Studies on the metabolism and toxicological detection of the new designer drugs, *N*-benzylpiperazine (*421*) and 4′-methyl-α-pyrrolidinopropiophenone (*422*), in urine have been done by GC/MS. Cathinone (Khat) and methcathinone (CAT) have been detected in urine specimens using GC/MS (*423*).

Drug recognition expert evaluations have been reviewed using limited data (*424*). Current blood level data have been presented for drugs and chemicals of toxicological interest (*425*).

Other Techniques. A review describing alternative strategies for postmortem drug testing has been published (*426*). Immunoassays have been used for the routine screening of drugs of abuse in biological fluids (*427*, *428*). Various extraction procedures for the analysis of drugs in urine and other biological fluids have been described (*429*−*434*). SPME has been applied to drug screening and identification (*435*−*438*). Advances in the use of mass spectral libraries for forensic toxicology have been reported (*439*, *440*). GC/MS has been used for various applications of the analysis of drugs of abuse (*441*−*445*). CE has been used for screening and identification of drugs of abuse in biological fluids (*446*, *447*). Advances in TLC for the analysis of drugs have been reported (*448*, *449*). The use of HPLC of basic drugs on microparticulate strong cation-exchange materials has been reviewed (*450*). Issues of when to use isotopically labeled analogues of analytes as internal standards in mass spectrometry have been addressed (*451*). The toxicological screening in urine for drugs by LC/TOF-MS with automated target library search based on elemental formulas has been presented (*452*). A screening method for psychoactive drugs and metabolites in human serum using SPE and HPLC/ESI-MS has been developed (*453*). IMS and GC/IMS have been applied to various aspects of drug screening (*454*−*457*). Chemometric detection of thermally degraded samples has been applied to the analysis of drugs of abuse with GC/FT-IR (*458*). Drugs of abuse have been analyzed by near-IR spectroscopy (*459*). Drugs of abuse have also been detected on U.S. paper currency (*460*), in hair (*461*), in perspiration (*462*, *463*), in oral fluid (*464*), in oral fluid and sweat wipes (*465*), in plasma, oral fluid, and sweat wipes (*466*), in bile (*467*), and in larvae of *Protophormia terraenovae* and *Calliphora vicina* (Diptera: Calliphoridae) (*468*). Web sources for use in forensic toxicology have been reviewed (*469*).

Thomas A. Brettell *is Director of the New Jersey State Police Forensic Science Laboratory Bureau. He received his B.A. degree (1973) in chemistry from Drew University, Madison, NJ; a M.S. degree (1975) in chemistry from Lehigh University, Bethlehem, PA; and Ph.D. degree (1987) in analytical chemistry from Villanova, University, Villanova, PA. Dr. Brettell joined the New Jersey State Police Forensic Science Bureau in 1976. He was awarded The Chromatography Forum of the Delaware Valley Award in 1997. Dr. Brettell presently teaches forensic science in the Law and Justice Departments of The College of New Jersey and Rider University. From 1984 to 1998 he was a member of the faculty of the New Jersey Governor's School in the Sciences. He is a past president of the Chromatography Forum of the Delaware Valley and has served on the A-Page Advisory Board of Analytical Chemistry. Dr. Brettell is a Fellow in the American Academy of Forensic Scientists (AAFS) and is the current Chairman of the Criminalistics Section of the AAFS. He is on the Board of Governors of the Eastern Analytical Symposium and is a certified Diplomate of the American Board of Criminalistics. He also holds memberships in the American Chemical Society, the New Jersey Association of Forensic Scientists, and the Northeastern Association of Forensic Scientists. His present research interests include headspace analysis in gas chromatography and Raman microspectroscopy.*

Norah Rudin *holds a B.A. from Pomona College and a Ph.D. from Brandeis University. She is a Diplomate of the American Board of Criminalistics, and a member of the California Association of Criminalists and the American Academy of Forensic Sciences. After completing a postdoctoral fellowship at Lawrence Berkeley Laboratory, she worked for three years as a full-time consultant for the California Department of Justice DNA Laboratory and has also served as consulting technical leader for the DNA programs at the Idaho Department of Law Enforcement DNA Laboratory, the San Francisco Crime Laboratory, and the San Diego County Sheriff's Department. Dr. Rudin divides her time between consulting, writing, and teaching about forensic DNA and forensic science as well as more general topics in biology. Dr. Rudin has coauthored An Introduction to DNA Forensic Analysis, a book that has become the preeminent reference for both attorneys and crime laboratories, and Principles and Practice of Criminalistics: The Profession of Forensic Science, a book aimed at practicing criminalists. She is also the author of Dictionary of Modern Biology. Dr. Rudin teaches a variety of general forensic and forensic DNA courses for the University of California at Berkeley extension and on-line. She is active as a consultant and expert witness in forensic DNA for both prosecution and defense.*

Richard Saferstein *is a forensic science consultant. He retired as Chief Forensic Scientist of the New Jersey State Police Laboratory in 1991. He received B.S. and M.A. degrees from the City College of New York in 1963 and 1966, respectively. He received his Ph.D. degree in chemistry from the City University of New York in 1970. Prior to his coming to the New Jersey State Police in 1970, he was employed as a forensic chemist with the Treasury Department (1964−1968) and served as an analytical chemist with Shell Chemical Co. (1969−1970). Dr. Saferstein is the author of a number of technical papers covering a variety of forensic topics. He has also written a book (8th edition) on the subject titled Criminalistics: An Introduction to Forensic Science (Prentice-Hall, 2004) and has edited Forensic Science Handbook, Vol. I, 2nd. ed., Vols. II and III (Prentice-Hall, 2002, 1988, 1993, respectively), and More Chemistry and Crime (Oxford University Press, 1997) reference texts dealing with important forensic science topics. Dr. Saferstein has served on the editorial boards of the Journal of Forensic Sciences, Journal of Applied and Analytical Pyrolysis, and Microchemical Journal. He currently serves on the editorial board of the Journal of Forensic Identification. He is a member of the American Chemical Society, the American Academy of Forensic Science, the American Microchemical Society, The Forensic Science Society, the Society of Forensic Toxicologists, the Canadian Society of Forensic Scientists, the International Association for Identification, the Northeastern Association of Forensic Scientists, the Northwestern Association of Forensic Scientists, and the Mid-Atlantic Association of Forensic Scientists.*

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