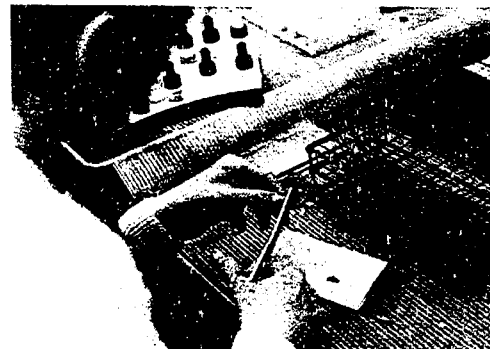
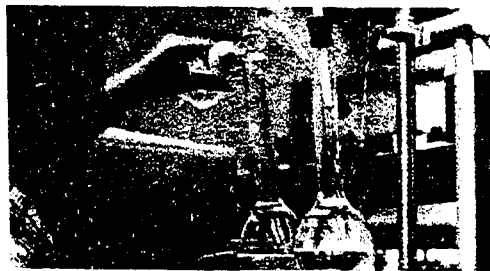


Sourcebook in Forensic Serology, Immunology, and Biochemistry



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National Institute of Justice

Sourcebook in Forensic Serology, Immunology, and Biochemistry

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with 1989 Update

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Sourcebook Errata

On page 17, the equation following line 11, after the words "The above equation may be rewritten:" should read as follows:

$$[ES] = \frac{k_1}{k_2 + k_3} [E][S]$$

The equation following lines 16 and 17, after the words "Solving for ES and substituting in the previous equation yields" should read as follows:

$$\frac{v_0}{V_{\max}} = \frac{k_1}{k_2 + k_3} \frac{[E] [S]}{[E_t]}$$

On page 426, lines 29 and 30 giving the correspondences of different PGM subtype nomenclatures should read as follows:

1- = a3 = 1F; 1+ = a1 = 1S; 2- = a4 = 2F; and 2+ = a2 = 2S.

PREFACE TO THE REPRINT EDITION

In the six years or so since this *Sourcebook* appeared, the stock of copies was exhausted. There were indications from a number of sources, however, that there was still a demand for copies and that the book still has a valuable place as a reference work in forensic biology. Accordingly, the National Institute of Justice is to be commended for its decision to reprint the book on a demand basis through the National Criminal Justice Reference Service. In this respect, I am grateful to Mr. James K. Stewart, Director of the Institute, and to Dr. Richard M. Rau, manager of the Forensic Science and Criminal Justice Technology Program. As a result of their continued support, this book will continue to be available to those interested in it.

It was not realistic to consider undertaking a complete and systematic update and review of all the literature that has appeared since the book's publication. I was asked, however, to provide a brief summary of some of the more recent information in the field in this new Preface, and that is its major purpose. Because the literature grows with such rapidity, a number of more recent reviews as well as some specific papers are cited here and an effort has been made to related these to specific subject areas covered in the book. In addition, an introduction to the rapidly developing field of molecular biology and DNA analysis that has recently become a part of forensic biology is given, and some references provided. DNA analysis has become a part of forensic serology in the relatively few years since the book was written.

Selected Recent Material on Sourcebook Subjects

Several more recent reviews cover blood and body fluid identification in stains, species determination and blood and body fluid stain grouping [1-3], and the application of genetic markers including HLA to parentage testing has also been reviewed [4-6]. Volume 1 of *Advances in Forensic Science* [7] contains a number of review chapters by noted authorities on various subjects: Divall, on menstrual blood identification (cf. §8.1); Katsumata and Oya, on feta and neonatal blood identification (cf. §§8.2 and 8.3); Suzuki and Oya on semen identification in stains (cf. §10); Fiori, on body fluid grouping (cf. §19.10.5); Benciolini and Cortivo, on ABO grouping of human hair (cf. §19.10.7.1); Carracedo, as well as Pascali, on isoelectric focusing and its applications in serum group protein typing (cf. §§ 40-43 and 45); Tumosa, on the occurrence of ABH antigens in infrahuman species; Newall, on typing HLA antigens in bloodstains (cf. §46.7.2.); and Smith, on detecting drugs in bloodstains (cf. §50.2.2).

In the area of semen identification, there have been a number of newer developments. The original description of and earlier papers on γ -seminoprotein (γ -Sm) are discussed in §10.10. Similarly, the original work on seminal protein p30 may be found in §10.14. It is highly likely (though perhaps not proven) that γ -Sm and p30 are identical to one another, as well as to "human prostate antigen" (sometimes abbreviated PA) [8]. The γ -Sm protein has been further characterized biochemically and its amino acid sequence determined [9-11]. An ELISA assay using anti- γ -Sm has been developed for use with seminal stains [12]. Similarly, an ELISA has been developed for p30 and shown to be applicable to the investigation of seminal stains and vaginal swabs in sexual assault cases [13].

Another smaller seminal protein of prostatic origin, called β -microseminoprotein (β -MSP) has been isolated and extensively characterized by Hara and his collaborators in Japan [14-17]. I am indebted to Prof. Dr. Mitsuwo Hara at the Kurume University School of Medicine for making copies of his more recent work available. Another human seminal protein of seminal vesicle origin, known as MHS-5, has been purified and a monoclonal antibody prepared against it [18]. The monoclonal anti-MHS-5 has been used as the basis for an ELISA test for human semen identification.

The theory underlying absorption-inhibition testing as well as a novel two-dimensional A-I method are discussed in a paper by Lee and collaborators [19]. More recent material on the biochemical genetics of and relationships between ABO, Lewis, Secretor and related antigens (cf. § 19.9) may be found in reviews by Watkins and by Oriol and coworkers [20-22]. Extensive reviews of the application of the polymorphic isoenzyme (and other) systems in forensic serology (cf. Unit VI) have been published by Sensabaugh [23-26].

The U.S. population data for various genetic marker systems that are included in the book have been updated and analyzed in a series of three papers [27-29]. In addition, several papers have discussed the application of population genetic marker data to stain typing information as might be obtained in particular case situations [24, 30-32].

The forthcoming third volume of *Advances in Forensic Science* [33] offers reviews of several important subjects, in addition to its extensive coverage of DNA typing (about which more below). Schanfield extensively reviews immunoglobulin allotyping (cf. §44), Bütler reviews and updates the Ag system (cf. §45.1.2), and Mayr reviews the application of HLA typing in disputed parentage cases (cf. §46.7.1).

In addition, Fletcher reviews enzyme-linked immunosorbent assay (ELISA) as applied to forensic blood and body fluid identification and grouping problems. ELISA applications have come along sufficiently recently that there is nothing about them in the book.

Molecular Biology and DNA Typing

Evidence that DNA is in fact the genetic material, the structure of the nucleic acids, and the manner in which DNA controls protein synthesis are briefly reviewed in §1.2.2 of this book. In the past decade or so, extraordinary advances have been made in the field of molecular biology. These advances have enabled the development of what is often called genetic engineering. Perhaps the most significant advances in molecular biology from the point of view of forensic biology have been: (1) the discovery and characterization of a large variety of restriction endonucleases and their widespread availability; (2) the discovery and refinement of techniques for cloning manageable-sized fragments of DNA into vectors; (3) the discovery of restriction fragment length polymorphisms and the availability of human DNA probes for their detection; and (4) the description and refinement of polymerase chain reaction techniques, and their use in connection with allele specific oligonucleotide probes.

The large array of restriction endonucleases (restriction enzymes; RE) allows very large DNA to be cleaved into smaller, manageable fragments for subsequent characterization and/or manipulation. Knowledge of the RE cleavage recognition sequences in DNA has meant that sequence information is available about the ends of the fragments produced.

Some of the REs produce blunt ends, but many others produce "jagged" cuts in double stranded DNA producing fragments in which a few bases from one strand protrude as a single strand beyond the terminus of the other strand. These few base single stranded ends are sometimes called "sticky," because if another piece of double stranded DNA with a complementary single stranded sticky end is produced, the two fragments of DNA can be recombined into a single double stranded molecule using appropriate ligases. Variations of this procedure form the basis of genetic engineering. Using these techniques, fragments of human DNA can be introduced into vectors (usually plasmids or cosmids). Then, by subsequent cloning, these human DNA fragments can be reproduced in any desirable quantity in perpetuity, and in addition they can be isolated and recovered from the vectors.

In recent years it has been recognized that the human genome contains substantial segments of repetitive sequence DNA [34,35]. Some repetitive DNA occurs in the form of relatively short, highly repeated sequences that have been called 'minisatellites.' Certain minisatellite loci have been found in the human genome at which there is substantial variation between individuals in the number of times the core sequence is repeated. If a RE is used to cleave DNA outside the repeat region, fragments of differing size are produced according to the number of repeats occurring in the region. Human DNA loci of this kind are termed "variable number of tandem repeat" or "VNTR" loci. Separation of the RE-digested VNTR fragments according to size by electrophoresis, transfer of the fragments to a nitrocellulose or nylon membrane, and hybridization with a labeled human DNA probe that recognizes the core sequence produces banding patterns that are characteristic of the individual from whom the DNA came.

This phenomenon is called "restriction fragment length polymorphism" or "RFLP" and is the basis of most current "DNA typing" as it is applied in forensic serology.

In 1980, Botstein, White, Skolnik and Davis [36] recognized that RFLP could be used as a basis for genetic mapping, and this approach has indeed yielded considerable information [37]. Wyman and White [38] soon described a highly polymorphic VNTR locus, and a large number of other such loci are now known. Not much notice of these developments was taken by the forensic science community until Jeffreys described several multilocus probes [39] that could be employed to produce what were described as DNA 'fingerprints' [40]. The importance of these findings in terms of their applications both to disputed parentage problems and to individual identification problems was quickly recognized [41,42].

In just the past few years, Jeffreys has further characterized the multilocus probes [43] and cloned a series of single locus probes recognizing several of the loci detected by the original probes [44-46]. These probes are used exclusively by Cellmark Diagnostics in their DNA typing work in the U.S. Other DNA probes are used by the Lifecodes Corp. in their DNA typing work, and some information about their probes and procedures has been published [47-49]. A number of DNA probes from GenMark are available through Promega, and still other probes are available from Collaborative Research, Inc. Recently, the FBI Laboratory initiated DNA typing in casework after a lengthy research and development effort aimed at selecting a typing system, appropriate probes, and validating the procedures that are to be used [50-51].

Another DNA analysis procedure that has already found limited application in forensic serology, and is certain to be significant in the future, is the polymerase chain reaction (PCR) technique. PCR was developed by Erlich and collaborators at the Cetus Corp. in California [52-53]. With PCR, specific sequences of DNA can be replicated to produce hundreds of thousands to millions of copies provided specific primers are available. The primers are constructed from knowledge of the sequences flanking the region of interest. PCR has been applied to the diagnosis of genetic disorders [54] and to the analysis of polymorphism at specific subregions of the *HLA* locus [55]. DNA analysis of the *HLA-DQ α* polymorphism has been described in single human hair roots [56]. Many samples of forensic interest are limited in quantity and may also have been subjected to environmental conditions that degrade the DNA. As a result, RFLP analysis may not be possible. PCR techniques are attractive for forensic analysis because so little DNA is required for analysis, and experience has shown that some samples which were unsuitable for RFLP analysis could be analyzed using PCR procedures. At the present time, PCR procedures are used in conjunction with allele-specific oligonucleotide (ASO) probes. The loci currently detected do not show the degree of polymorphism exhibited by RFLP loci. The information obtained at present from PCR analysis is thus very valuable as an exclusionary tool, but less valuable in inclusionary cases. Efforts are underway in many laboratories, however, to develop primers that will enable PCR amplification of VNTR loci [57]. Further research and development will be necessary to evaluate the forensic applications of PCR techniques, as very few forensic laboratories have had much experience with PCR at the present time.

Further information about DNA typing and its forensic applications may be obtained from a number of currently available sources. Volume 3 of *Advances in Forensic Science* [33] will have ~~eight~~^{seven} chapters on DNA polymorphisms and their forensic applications. The Banbury Conference on forensic applications of DNA has papers by a number of authorities in the field [58]. The FBI Forensic Science Research and Training Center at Quantico has sponsored two major symposia on the forensic applications of DNA [59,60]. The proceedings of the first of these symposia are available on videotape, and the proceedings of the second will be published. An extensive report on forensic applications of DNA typing is currently in preparation by the Office of Technology Assessment of the U.S. Congress, and should be delivered sometime in 1989.

DNA typing is undoubtedly the most exciting development in forensic serology in many years, and arguably the most exciting development ever. It will take some time for techniques and procedures to be worked out and tested on a relatively wide scale. The need for standardization of methodology and for some general agreement on procedures for the interpretation of RFLP typing results has recently been discussed [58,61]. In the next few years, molecular and forensic biologists working together will undoubtedly establish guidelines and standards for reliable and reproducible DNA typing procedures that can be widely employed in the analysis of both disputed parentage and identification cases.

West Haven, CT
July, 1989

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FOREWORD

The National Institute of Justice is pleased to publish this important reference work for forensic serologists. The late John O. Sullivan, manager of the Institute's forensic science program from 1975 to 1981, played a key role in encouraging and supporting development of this publication. It is a particularly fitting legacy of Mr. Sullivan's contributions to advancing the state of the art in the forensic sciences.

James K. Stewart
Director

PREFACE

For a number of years, I have thought it would be desirable to have available a comprehensive review of the literature of the many subjects that now comprise forensic serology, immunology and biochemistry. My appointment as a Visiting Fellow in the National Institute of Law Enforcement and Criminal Justice (now the National Institute of Justice) in 1976 afforded me the opportunity to prepare this review. I trust that the product may be a useful reference work for forensic serologists working in various laboratories, particularly in this country.

I have taken a more or less historical approach to each of the major subjects, in part because I thought it would provide continuity, and in part because I thought it would be more interesting. Accordingly, the different subject areas are discussed from the time of their origins in the published literature up to the present time. Much of the material is now of purely historical interest, and does not represent the current understanding of the subjects. I hope that the distinctions between older notions of purely historical interest, and current ones, have been clearly made.

There are many excellent reviews of the subjects covered here by specialists in those fields. They treat the various topics more comprehensively and better than I have been able to do, and I have cited them in the reference lists. In this work, I have attempted to treat all the subjects of interest in present-day forensic serology, and to combine the historical developments, the essential background information, and the forensic applications under the same cover.

This work has been entitled a "sourcebook", because it is quite simply a narrative review of the scientific literature. Because I regard this book primarily as a guide to the published literature, careful attention has been paid to the accuracy of the reference lists which appear at the end of each unit.

The book is divided into a total of nine units. The first unit consists of background material in serology, immunology, biochemistry, genetics and methods that are employed in the field. I was persuaded that this material should be included, and that it might serve a useful purpose. Units II and III have to do with the identification of blood and body fluids, respectively, and Unit IV has to do with species determination. These make up most of the identification sections. Units V, VI and VII have to do with the different classes of genetic markers in blood and body fluids, and make up most of the individualization sections. Unit VIII is concerned with the sexing of bloodstains, and with efforts to individualize blood using non-genetic markers. Unit IX consists of a set of translations of original papers of historical interest in the field. The rationale for the translations set is discussed in the Preface to that unit, which is self-contained. The eight units of the sourcebook are further divided into sections and subsections.

References are compiled at the end of each unit. Because of the large number of references, some consistent bibliographic style had to be selected, and in arriving at these conventions, I have made an effort to provide as much information as possible for readers who wish to find particular references. An effort was made to consult every reference which is cited here. References which could not be examined have a notation of the source that was used. These are indicated as "cited by" or "through". If another reference contains similar information to the one cited, or an abstract of it, I have indicated this fact with the words "and see".

The A.I.B.S. convention has been followed in citing all the references [Council of Biological Editors, Committee on Form and Style: *CBE Style Manual*, 3rd ed., American Institute of Biological Sciences, Washington, D.C., 1972]. References are cited in the text by the name(s) of the author(s) and the year the paper was published. The use of the name(s) and year as part of a sentence constitutes a citation. Papers written by more than two authors are cited in the text by the last name of the first author, and "*et al.*", followed by the year. In cases where the same author(s) wrote

several papers in the same year, they are distinguished in the text and in the reference lists by lower case arabic letters, e.g. 1971a, 1971b, etc. In some cases, a senior author with two or more coauthors, not always the same people, wrote more than one paper in a given year. The year and lower case letter convention is used to distinguish these, even though the full list of names on the papers is not the same. Thus, for example, if A. Smith, B. Jones and C. Williams wrote a paper in 1960, and A. Smith, B. Jones, C. Johnson and D. Williams had another paper in the same year, the former would be cited in the text as "Smith *et al.*, 1960a", the latter as "Smith *et al.*, 1960b". The arabic letters are used in the reference list as well as in the text in these cases. The reference lists are in strict alphabetical order by first letter of last name of first author, including institutional authors. Editorials are cited as "Editorial", unless they were signed, and it was clear who wrote them. In the older literature, first name(s) or initial(s) of authors were not always given. There was a tendency to use titles. Authors' initials which are given in parentheses in the reference lists were supplied, and did not appear in the original article. Titles of articles are given in full in the original language, except in cases where the original language does not use the Latinic alphabet. I have tried to retain accent and diacritical marks in citing authors' names and article titles. Russian and Japanese journals generally provide an English translation of the names of authors and the title of the article. I have usually given these in English. Transliteration of author names and article titles from sources in languages using Cyrillic alphabets follow the *U.S. Government Printing Office Style Manual* (1973). Abbreviations of journal titles have been taken from *Bibliographic Guide for Editors and Authors*, American Chemical Society, Washington, D.C., 1974, or from *BIOSIS List of Serials*, BioSciences Information Service of Biological Abstracts, Philadelphia, PA, 1976. In cases where these sources did not provide a standard abbreviation, I have followed the guidelines given in ANSI Standard Z39.5-1969 (R1974) of the American National Standards Institute in arriving at the usage which appears.

In some libraries, foreign journals are catalogued according to their foreign titles. Where I encountered this practice, footnotes were added to the reference lists giving the appropriate information. Similarly, many journals have undergone title changes over the years, many have been superseded by other journals, and some have been divided up into a number of separate parts, and so forth. In cases where I thought these changes might cause difficulty in locating an article in a library, I have added explanatory bibliographic footnotes. The principal Russian medicolegal journal Судебно-медицинская Экспертиза is uniformly cited in the reference lists as "Sud. Med. Ekspert.". Journal title abbreviations are set in italic type, and volume numbers are in boldface type. In many cases, journals have been issued in several series over the years. Sometimes, the original volume numbering was dropped when a new series was issued, but in other cases it was retained. The series in which the cited volume number appeared is given in parentheses following the volume number. "N.S." means "new series" and this series is always the second one. In German language journals, the word "Folge" indicates a series; thus, "N.F." means "neue Folge", "3F" means third "Folge", and so on. If the original volume number was retained in the journal, even though a subsequent series designation was being used, both designations are given. For example, "21 (2 ser. 6)" means that the piece is the 21st volume of the journal, and is also the 6th volume of the second series. An arabic numeral in parentheses following the volume number is the *number* of the journal within the particular volume (or over-all). Thus, "14(12)" indicates volume 14, number 12. I included this in some cases because it was common in the older literature to cite references by number only, rather than by volume and page number. Thus an author might cite "*Berl. Klin. Wochenschr.*, 1906, No. 6". I would cite this reference as "*Berl. Klin. Wochenschr.* 43(6): pages". In this way, a reader could verify that the two papers were the same, though cited differently. Full pagination for each article has been given as called for by the A.I.B.S. convention. A single page number indicates that the reference occupies only one page. Deviations from these conventions are in the direction of giving more information about the reference. I hope that the use of well defined conventions, and explanatory footnotes where they seem to be necessary, will help readers to find references in which they are interested more easily than I was able to do in many cases.

Papers in the reference lists marked with the symbol ¶ have been translated into English as part of the translations set, which appears as Unit IX.

The term "substrate" is sometimes used in forensic serology to mean the object or material upon which a stain was deposited. The term also has the technical biochemical meaning of the reactant(s) in enzyme-catalyzed reactions. I have restricted the use of "substrate" to the biochemical meaning. Objects or materials upon which stains have been deposited are "substrata" (singular: "substratum").

In many of the respective sections dealing with genetic marker systems, I have compiled as much U.S. population data as I could find with a reasonable amount of effort. Some criteria had to be used in selecting and presenting this data. Since this book was prepared with forensic serologists in this country in mind, I have included only U.S. population data. I also decided, arbitrarily, not to include any data published before 1950. The data are presented in tables in essentially the same form as given by the original author(s). The only additions I have made are percentages of individuals representing various phenotypes, in cases where the author(s) gave only numbers. I have not tried to calculate numbers if the author(s) presented percentages. The population sampled is described in the terms used by the original author(s). At the present time, the single, most comprehensive reference work on population data ever compiled is the 1976 edition of *The Distribution of the Human Blood Groups and Other Polymorphisms*, by Mourant, Kopeć and Domaniewska-Sobczak (cited in the text as Mourant *et al.*, 1976). No one seriously interested in human blood group population data can do without this reference. In the older literature, a comprehensive tabulation was prepared by W.C. Boyd in 1939. ABO and MN frequencies for many of the world's populations which had been studied were given.

Because this book took considerably longer to complete than was originally anticipated, some more recent references may be cited in later units, and not in earlier ones, even though they contain information on the subjects covered in both places. I have made some effort to remedy this problem in revision, but may not have succeeded entirely.

A large number of people have been helpful to me in many different ways in the course of this project. I take pleasure in acknowledging their help and assistance in the remainder of the preface. I am grateful to the following for granting their kind permission to use material from figures and tables in published sources: American Association for the Advancement of Science [publishers of *Science*]; Dr. V. A. McKusick; W. B. Saunders & Co.; Elsevier Sequoia, S.A.; Prof. Dr. Hiroshi Hirose in Japan; Interscience Publishers, Division of John Wiley & Sons; and Rutgers University Press.

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As the project was largely bibliographic, I could not have managed it without the assistance of many people associated with various libraries I used. Morton Goren and Lavonne Wienke of the LEAA Library were very helpful in obtaining interlibrary loan materials. I owe a special debt of thanks to Mr. Albert Berkowitz and his staff at the National Library of Medicine in Bethesda. They provided me with space to work, and a most congenial environment in which to do so, for more than two years time. The NLM staff treated me as a colleague during my stay in the library. I would particularly thank Doralee Agayoff, Jeanne Crosier, Edith Blair, Paula Strain, Maxine Henke, Peggy Beavers, Richard Mumford, John Broadwyn, Dr. Stephen Kim, Charlotte Kenton, and Dorothy Hanks. All of them went out of their way to assist me in

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I reserve very special praise and gratitude for Maureen Swift and for Danice Gomien. Ms. Swift typed the entire manuscript, in some cases more than once. Ms. Gomien prepared all the figures and tables. Both of them navigated hundreds of pages of difficult material skillfully and well, somehow managing to make sense out of the many curious symbols and usages that are found in this field. There is no doubt that the work could not have been completed but for their continuing cooperation and perseverance.

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