Forensic Analysis of Biological Evidence

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Chapter 29. Forensic Analysis of Biological Evidence

[The original 1982 version of this chapter and the 1989 revision were entitled "Forensic Serology: Analysis of Bloodstains and Body Fluid Stains:" A coauthor of the original chapter was Frank R. Camp, Jr., Col., U.S. Army (Ret.) (1919-1983). Col. Camp was a scientist, military officer, blood banker, and historian of science, as well as a mentor and friend. to many. In his 32-year military career, Col. Camp became the foremost authority on blood banking and immunohematology in the United States military services. More than 200 scientific papers, chapters, technical and scientific reports, and translations of classical papers in blood grouping and immunohematology bear his name as author or coauthor. For 12 years, he was Commander/Director of the U.S. Army Medical Research Laboratories at Fort Knox, Kentucky, presiding over that facility's development as a major research, training, and service center for blood banking and immunohematology for the military services. Following retirement from active duty, Col. Camp became Scientific Director! Director of the American Red Cross Blood Services Regional Center in Louisville, Kentucky. During his career, Col. Camp held teaching appointments at the Bowling Green State University (Ohio) and the University of Louisville School of Medicine. Col. Camp was the recipient of many awards and honors, including the Distinguished Service Award of the AABB and the Legion of Merit Medal. In 1970, he was awarded the "A" prefix to his military occupational specialty by the U.S. Army Surgeon General. He was a member of more than 30 professional and scientific societies and organizations. On July 1, 1984, the Blood Bank Center at Fort Knox became the Col. Frank R. Camp Memorial Blood Center-a permanent tribute to the man and to his accomplishments and legacy. A memoriam to Col. Camp appears at 81 J. Ky. Med. Assn 740 (1983).]

SYNOPSIS

FORENSIC ANALYSIS OF BIOLOGICAL EVIDENCE

§ 29.01 Development of Forensic Serology-From Genetic-Marker Systems to DNA Typing

The term "forensic serology" has generally been used to refer to the identification and individualization of biological evidence, including all the activities and tests associated with the evaluation and typing of biological evidence in criminal matters. The word "serology" derived from serum, the fraction of blood containing antibodies. Blood grouping was long the only means of individualizing biological evidence, and "serology" classically encompassed blood groups and blood grouping. Over time, the terminology used to describe these activities has changed and perhaps become a bit confusing.

Forensic techniques for typing deoxyribonucleic acid (DNA),[1] which began developing around 1985, have replaced the classical or traditional genetic systems previously used, so that "forensic serology" is no longer a good descriptor of biological evidence analysis units or their activities. Some laboratories now use terms like "forensic biology" for this purpose. "Forensic biology" is sometimes divided into DNA typing, which may be called "forensic molecular biology," and the preliminary analysis activities (e.g., evaluation of evidence, identification, species, selection of specimens for typing), which may, be called "forensic biochemistry" and are sometimes still referred to as "forensic serology." Use of the term "forensic biology" to refer to blood and body fluid evidence analysis is unfortunate because it fails to distinguish other legitimate kinds of forensic biological analyses, such as those involving botany or palynology.

Forensic serology has been a major area of forensic science for many decades. It evolved in the late nineteenth and early twentieth centuries, primarily in medicolegal institutes in Europe and the United States. In the United States, medicolegal institutes disappeared around the time of the First World War. Soon afterward, in the late 1920s and early 1930s, forensic science laboratories began to develop within law enforcement agencies, and forensic serology was, and still is, a major focus of the activities of those laboratories.[2]

Forensic serology has traditionally been the area of criminalistics that deals with biological evidence. Generally, criminalistics is the evaluation of physical evidence in matters of legal interest for purposes of providing scientific information to triers of fact. Areas encompassed within the general field of criminalistics include serology, forensic chemistry, trace evidence analysis, microscopy, and some types of reconstructions. Criminalistics is generally distinguished as separate from forensic medicine, odontology, anthropology, toxicology, engineering, psychiatry, and is often distinguished from questioned documents, firearms and tool marks, and fingerprint and other impression examinations. The three principal activities encompassed within criminalistics are identification, individualization, and reconstruction.[3]

Identification in this context means establishing the identity of a substance or material. In biological evidence examinations, identification tests are designed to determine the nature of a questioned material, such as blood, semen, or saliva. Identification tests are also used to determine the species of origin of blood (human, animal, etc.). In some cases, tests may be performed to try to show that human blood originated from a particular source (e.g., menstrual).

Individualization of physical evidence involves the determination that an item is unique among a particular class of items. True individualization is not possible with most types of physical evidence. In biological evidence analysis, individualization means that a blood or body fluid specimen can be shown to have come from a single individual. DNA typing has reached the stage at which effective individualization is possible (there is at least a scientifically justifiable statistical basis for individuality). Before DNA typing, biological evidence could not truly be individualized, although it could be partially individualized because some fraction of the population could be excluded as possible donors. The degree of individualization (the size of the fraction of the population that could be excluded or included) varied according to how many genetic systems were typed and what types were observed.

Reconstruction involves the use of physical evidence analysis to try to decide what actually happened in a given situation after the fact. Biological evidence analysis can contribute to the reconstruction of events in some cases. The interpretation of blood or body fluid stain patterns can be helpful, although bloodstain pattern interpretation is generally considered a separate specialty.

Forensic serology has traditionally been concerned with the identification and individualization of biological evidence, and methods have been adapted from histology, microscopy, immunology, biochemistry, and serology for those purposes. The classical serological and biochemical genetic markers are no longer used for biological evidence individualization, having been supplanted entirely by DNA typing. Accordingly, this chapter focuses on the identification aspects of biological evidence analysis. The classical or traditional genetic markers are described and reviewed briefly as a concession to historical continuity and because questions still occasionally arise in older cases from the pre-DNA era.

In the United States, parentage testing[4] developed in clinical laboratories and has long been closely associated with blood banking, clinical medicine, and histocompatibility testing. The parentage testing community has always been separate from the forensic serology community, but with some notable exceptions, the two fields have long used very similar methods and systems to individualize blood. Like stain analysis, parentage testing is now also done exclusively by DNA typing.

The material covered in this chapter has been extensively reviewed elsewhere. A number of helpful references are cited throughout the chapter, and those works can be consulted for additional and often more detailed information.[5]

§ 29.02 Scope of Forensic Biological Evidence Analysis

In criminal matters, particularly those involving violence, specimens of blood, semen, and other body fluids or tissues can be analyzed for identification purposes and then individualized by deoxyribonucleic acid (DNA) typing. The DNA types of persons involved in the criminal case can be determined and compared with the types obtained from the case specimens. With multiple-locus RFLP (restriction fragment length polymorphism) or STR (short tandem repeat) DNA typing, the probabilities of a chance match are sufficiently low that specimens are effectively individualized in match cases. Correspondingly, there is virtually no chance a non-depositor would fail to be excluded using the same methods. Cases involving homicide, assault, and sexual assault are the most commonly encountered in the biological evidence examination units of forensic science laboratories.

The identification and species-determination aspects of a forensic examination can sometimes be more important to a case than DNA typing. For example, a suspected hit-and-run driver might be absolved of suspicion by a finding that bloodstains on his vehicle were of nonhuman origin. In contrast, a virtual match between evidence and a person might have little meaning if there is an innocent explanation for the finding. This would be true, for example, when a victim's genetic profile is matched to bloodstains on a suspect's clothing in a case in which the victim and suspect lived in the same household or the suspect is able to offer a plausible explanation for the stains.

Conventional disputed parentage testing is rarely if ever done in forensic science laboratories, but there are situations in which parentage-testing methods can be used to identify bloodstains or other nondescript remains from a missing person. Similarly, such methods might be used to help establish a party's claim to an estate through genetic affiliation. Mitochondrial DNA sequencing analysis can be used to help establish identity in cases involving human remains and can sometimes be used to associate or disassociate telogen hairs with a particular person. This subject is discussed further in Chapter 37C, DNA Typing-Criminal and Civil Applications.

§ 29.03 Characteristics of Blood and Physiological Fluids

[a] Blood

Blood is a very complicated liquid tissue. It serves as the transporting medium for all the substances in the body. Blood has two major types of components-the cellular elements and the liquid portion. Blood can be prevented from clotting by the addition of a chemical called an anticoagulant. -If a tube of blood containing an anticoagulant is allowed to sit still for a time, it will be seen to separate into two parts. The liquid portion has a yellowish color and is usually transparent if the blood came from a healthy person. This liquid part is called plasma. If a tube of blood without anticoagulant added is allowed to clot, the liquid part that forms is called serum. The distinction between plasma and serum is not particularly important in forensic serology, and the two terms can be used interchangeably in most contexts. The part of the blood that settles to the bottom of the tube has a deep red color and constitutes the cellular fraction. Three kinds of cells are found in the cellular fraction: (1) red blood cells, or erythrocytes; (2) white blood cells, or leucocytes (of which there are a number of different kinds); and (3) platelets, or thrombocytes. Red blood cells get their color (and their name) because they contain large amounts of hemoglobin. Hemoglobin is the oxygen-transporting protein of blood that is essential for life. Leucocytes, or white blood cells, perform complex functions having to do with immunity against diseases and infections. Platelets are involved in blood clotting. The numbers of red cells and white cells in a person's blood, the amount of hemoglobin, and the percentage of total blood volume occupied by cellular elements (hematocrit) are important indicators of health or disease. The blood cells are sometimes called "formed elements."

Plasma (serum) contains many proteins in addition to water, electrolytes, and other biochemicals. Albumin and immunoglobulins are important components because they tend to be species-specific and serve as the basis for immunological species tests. Plasma also contains a number of proteins that show genetic variation in populations. These were used as genetic markers for a number of years, as described further in § 29.07 below. Some properties of blood and normal values and ranges for some of its constituents are shown in Table 29-1.

Table 29-1. Some Components and Properties of Human Blood

Component/Property	**Normal Value/Range**	**Genetic Marker Class *****
Erythrocytes (Red Cells)	Male: 4.6-6.2 million/μL* Female: 4.6-5:4 million/μL	Blood groups Isoenzymes
Hemoglobin	Male: 13.5-18.0 g/dL ** Female: 12.0-16.0 g/dL	Hemoglobin variants
Leucocytes (White Cells)	4.5-11.0 thousand/μL	HLA antigens DNA
Platelets	150-440 thousand/μL	
Hematocrit	Male: 40-54% Female: 38-47%	
Plasma (Serum)		Serum groups

* Microliters (one-millionth of a liter).
** Grams per deciliter (grams per 0.1 liter).
*** The classes of traditional genetic markers are: blood groups; isoenzymes; serum groups; hemoglobin variants; and HLA. DNA in blood comes from the white blood cells.

[b] Semen, Saliva, Urine, and Other Physiological Fluids

The physiological fluids primarily found in forensic cases are semen, saliva, and urine. Other physiological fluids, including sweat, fecal matter, and gastric fluid, are seen less frequently.

The most commonly encountered physiological fluid in forensic science laboratories is semen, primarily because of sexual assault cases. Semen consists of two components -- spermatozoa and seminal fluid (seminal plasma). Spermatozoa are the male reproductive cells, and a normal human ejaculate can contain from several million upward to 80 million sperm cells per mL. Identification of spermatozoa is a primary means of semen identification. Seminal plasma provides the fluid environment for the spermatozoa. It contains inorganic salts, small molecules, and proteins, a number of which have served as bases for identification tests over the years.

Saliva, which is found in the mouth, is a mixture of secretions by the three salivary glands-the parotid, the sublingual, and the submaxillary. The composition of saliva is complex, and many proteins in saliva are thought to play a role in oral health and possibly in the prevention of dental decay and disease.

Urine is liquid human waste, containing water, salts, and a variety of small molecules. Urine is occasionally encountered in forensic cases, often in the form of dried stains.

§ 29.04 Blood Identification

Questioned biological specimens, often found in the form of dried stains, are always subjected to identification tests as the first step in a forensic examination. If blood is identified, tests are then performed to see whether the blood is of human origin. Once a specimen is known to contain human blood, it is typically subjected to genetic typing. If the blood is not human, further tests may be required to determine its species of origin.

There are generally two classes of identification tests-presumptive and confirmatory. Presumptive tests are usually quick and often quite sensitive, but not specific. They are used as rapid, preliminary screening tests and for searching evidence items, and positive results indicate the need for confirmatory testing. A positive confirmatory test is required to identify blood rigorously.

All tests for the identification of blood are based on the detection of hemoglobin. Hemoglobin consists of a heme moiety, four copies of which are associated with the globin protein moiety in am intact hemoglobin molecule. The classical presumptive and crystal tests are based on the detection of heme or of heme derivatives (modified heme that is chemically different from that in fresh blood). The derivatives may be present in the sample as a natural result of drying and aging, or they may be prepared intentionally as the basis of the test being used.

[a] Presumptive Tests

All presumptive blood tests in common use are catalytic tests, a designation that derives from the fact that they are all based on the catalytic activity of the heme in hemoglobin. Hemoglobin is not itself an enzyme (biological catalyst) in the body, but the molecule has considerable peroxidase activity, which can be demonstrated in the test tube using a variety of oxidizable substrates. A "substrate" is an organic molecule that can undergo a chemical reaction. Reactions can be catalyzed (speeded up) by an enzyme catalyst. The substrates used in these tests can all undergo oxidation reactions with hydrogen peroxide. A "peroxidase" is an enzyme (protein catalyst) that can 'speed up these oxidation reactions, and the heme portion of hemoglobin acts like a "peroxidase" in these tests.

Presumptive tests are designed to be quick and convenient, so the substrates chosen are those that give an easily recognizable color change. The catalytic tests are very sensitive, but not entirely specific. False positive results are possible, and positive catalytic test results require that a confirmatory test be carried out in order to be certain that the tested specimen is blood. A summary of catalytic tests and the chemicals used in each of them is given in Table 29-2, in § 29.04[b] below.

One reagent that has been used as the basis for presumptive blood testing is 3-aminophthalhydrazide, commonly called luminol. Luminol does not work in the same way as the other test reagents. Under certain conditions, luminol is chemiluminescent while undergoing oxidation in alkaline solution: Luminol is used most commonly at crime scenes to reveal bloodstains that are .not readily, apparent to the naked eye. Most laboratories use phenolphthalin; tolidine, tetramethylbenzidine, or leucomalachite green for preliminary blood identification testing.

[b] Confirmatory Tests

There are two types of confirmatory tests--crystal tests and anti-human hemoglobin tests. The crystal tests are the oldest tests for blood, the first having been described in 1853. They were used for many decades, but are no longer common. Many laboratories rely on immunological tests with anti-human hemoglobin to confirm the presence of blood. This practice has the advantage of giving species of origin information as well. In addition, some of the conventional genetic-marker typing results can provide unequivocal evidence that a specimen is blood, and even in some cases that it is human.

First proposed in 1905, anti-human hemoglobin tests have the same underlying scientific basis as species of origin tests (see § 29.05 below). A great advantage of this procedure is that an identification test for blood and a human species test are combined in a single operation. In order to realize this advantage, however, the anti-human hemoglobin reagent must be species-specific. Many anti-human hemoglobin reagents are at least relatively species-specific (primate-specific; for example). These antisera may be regarded as human-specific to the extent that nonhuman primates can be excluded as possible sources of a specimen.

Commercial sources of anti-human hemoglobin sera with consistent properties have not been widely available, at least for polyclonal antisera. The situation is better if laboratories are able to use monoclonal antibodies. The latter requires that laboratories have validated enzyme-linked immunosorbent assay (ELISA) tests available.

Table 29-2. Identification Tests for Blood

Presumptive Catalytic Tests	
Reagent and/or Name of Test	**Original Author(s), Year ***
Guaiacum / Van Deen's'Test	Van Deen, 1862
Day's Test	Day, 1867
Aloin	Klunge, 1882
Phenolphthalin / Kastle-Meyer Test	Kastle & Shedd, 1901; Meyer, 1903; Utz, 1903; Higaki and Philp, 1976
Benzidine / Adlers' Test	Adler & Adler, 1904
Leucomalachite Green	Adler & Adler, 1904
o-Tolidine	Ruttan & Hardisty, 1912
o-Toluidine	Gershenfeld, 1939
o-Dianisidine	Owen et al., 1958
Tetramethylbenzidine (TMB)	Holland et al., 1974; Garner et al., 1976
Luminol	Specht, 1937
Fluorescin	Lee et al., 1979
Confirmatory Crystal Tests	
Reagent and/or Name of Test	**Original Author(s), Year ***
Hematin halide / Teichmann's Test	Teichmann, 1853
Pyridine hemochromogen / Takayama's Test	Takayama, 1912
Acetone Chlorhemin / Modified Teichmann	Wagenaar, 1935
Confirmatory Anti-Human Hemoglobin Tests	Klein, 1905; Hektoen & Schulhof, 1923; Heidelberger & Landsteiner, 1923; Baxter & Rees, 1974

* References can be found in R.E. Gaensslen, Sourcebook in Forensic Serology, Immunology and Biochemistry (U.S. Government Printing Office, Washington D.C. 1983).

Once in a while, it is necessary to be able to distinguish between menstrual blood and ordinary circulation blood. A number of methods have been proposed over the years for identifying menstrual blood. The most useful methods are based on the fact that menstrual blood does not clot because it has an active fibrinolytic system (this system is the antagonist of the clotting system), and thus menstrual blood exhibits what is called "fibrinolytic activity," i.e., it will cause the digestion of fibrin protein. This property can be used as the basis of medicolegal tests for menstrual blood identification.[6] Generally speaking, positive results using these methods under properly controlled conditions permit an analyst to diagnose menstrual blood in contrast to circulation blood. However, false negative results are possible, so that negative test results cannot be taken to mean that menstrual blood is absent.

§ 29.05 Species Determination of Blood

[a] Obsolete Methods

Before 1900, species determination of dried blood was an extremely difficult problem. In general, the problem was approached by attempting to measure the average size of a statistically significant number of blood cells recovered from a stain. Quite apart from the intrinsic difficulties of such measurements and the fact that red blood cell sizes among many mammalian species are very similar, the entire approach rested on the faulty assumption that intact red cells, identical to the original ones, could be recovered from dried stains. Prevost and Dumas, in 1821, first pointed out the apparent size differences,[7] and the subject was subsequently pursued by many workers engaged in bloodstain examination, who conducted extensive studies on the subject.[8] Although some became convinced of the value of the method, others were doubtful. The method was employed nonetheless in criminal cases in the United States and elsewhere.

[b] Immunological Species Tests

The "precipitin test" and the "anti-human hemoglobin" test are both immunological procedures. Their underlying scientific principles are the same. All laboratories now use immunological methods to test for species of origin.

Higher animals have an immune system that enables them to make antibodies against "foreign" substances. Antibodies are proteins, and they are very specific; they react only with the substances that caused their formation in the first place (or with very closely related substances). The substances that cause antibody formation are called antigens. Before an animal will make antibodies against a substance, its immunological system has to recognize that the substance is "foreign," that is, not a part of the animal's own makeup.

If human serum, which contains many proteins, is injected into a rabbit, the rabbit's system will recognize the proteins as "foreign" and will begin to make antibodies against the human serum proteins. These antibodies are made by the rabbit's white blood cells and are then released into its serum. If the rabbit is bled and its serum collected, the antibodies will react specifically when mixed with human proteins by forming precipitates -- i.e., large aggregates of human protein and antibody molecules, which fall out of solution (precipitate). Accordingly, the rabbit serum antibodies are called "precipitins." The rabbit serum containing precipitin antibodies against human proteins is called an "antiserum." The particular serum described above would be called a "rabbit anti-human serum," meaning a rabbit serum containing antibodies against human proteins. Antisera can be made in many different animals, but rabbits and goats are the most common.

The immunological discoveries of the last decade of the nineteenth century laid the groundwork for the exploitation of immunological species specificity in forensic work. Myers and Uhlenhuth independently noted that precipitating antibodies raised in rabbits would specifically distinguish the egg albumins of various species of birds,[9] and it was a short step from those results to the preparation of species-specific antisera against blood proteins. The breakthrough came in 1901, when almost simultaneously, Uhlenhuth, in Greifswald, and Wassermann and Schuetze, in Berlin, independently reported that precipitating antibodies against the blood or serum of an animal would reliably and specifically detect the animal's blood in a dried stain.[10] The "precipitin test," as it is often called, was rapidly adopted by forensic workers.

This test was valuable not only for diagnosing human blood, but also in distinguishing the blood of game animals in game law enforcement. Using specific precipitating antisera, Gay solved an illegal deer killing case in Massachusetts in 1908, and Clarke solved a similar case in California in 1914.[11] Uhlenhuth continued to work on forensic applications of the precipitin test for many years. In 1909, he published an extensive treatise on the subject with Weidanz, and eight years before his death in 1957, Uhlenhuth published an interesting memoir of his personal recollections of the development of precipitin procedures.[12]

[1] Test Methods

Precipitin tests can be performed in various ways. In every instance, however, the goal is to bring the antigens and antibodies, both in solution, into contact with one another.

The earliest precipitin test method was the so-called "ring test." In this type of test, an aqueous solution of antigen is layered over the more dense antiserum solution in a tube. The precipitate forms at the interface between the layers in a positive test. This method was largely abandoned when gel-based methods were developed, in part because it required considerably more antiserum and antigen solution.

Gel diffusion methods have been commonly used for many years in the detection of antigen-antibody precipitin reactions. The single immunodiffusion technique first described by Oudin in 1946 has since been refined to a high degree and is widely used for certain types of analysis.[13] So-called "double diffusion," described by Ouchterlony and Elek, is preferred by many, since much wider latitude in the antigen and antibody concentrations is possible without seriously affecting the result.[14] This technique has also been refined and can be adapted to small amounts of material. Double immunodiffusion technique is used in forensic laboratories for species (and other) immunological tests. It has an advantage over other techniques, namely that information about the relationship between different antigens using an antibody to one of them can be discerned from the test results. This feature can be helpful in interpretation.

When conducting any of the immunodiffusion tests, agarose gels are prepared in small dishes or containers. Agarose is a purified; colloidal, semi-transparent substance made from seaweed. It can be liquefied at higher temperatures and then forms a solid gel when it is cooled down. The hot liquefied agarose can be poured into a container and allowed to cool to form a gel. Small holes can then be punched into the gel and filled with antiserum and antigen-solution. As the antibody molecules in the antiserum and the antigen molecules in the test solution diffuse through the gel, they contact one another and form a precipitin line in the gel that is visible in a positive test. Diffusion of the molecules in the gel is a slow process.

The process of electrophoresis can be used to speed up the migration of antigen and antibody molecules in a gel. Electrophoresis in effect amounts to applying an electric current to opposite sides of a gel. The antibodies and antigens, which are charged positively or negatively, then migrate due to the electric field. This procedure, called "crossed over electrophoresis," can speed up the precipitin test and save some time. It also requires less antibody and antigen than double diffusion. First described by Bussard in 1959 and adapted and refined for forensic blood testing by Culliford, the procedure has the advantages of speed, economy of material, and the ability to test many samples simultaneously.[15] The choice of agar to be used is critical for crossed over electrophoresis, since proper electroendosmotic properties of the medium are essential to the success of the method.

Although somewhat faster and requiring smaller quantities of material, crossed over electrophoresis cannot provide information on the relationships of antigens using antibodies to one of them, as double diffusion can.

[2] Technical Issues and Interpretation of Results

The two principal concerns in carrying out proper precipitin tests for the identification of species of origin are: (1) a proper relationship between the concentrations of antigen arid antibody; and (2) an appreciation of the cross-reactivity of antisera against one species with bloods of closely related species. Both of these factors are dependent to a certain extent on the particular antiserum being used. New batches of antisera should be tested against homologous (same species) blood and bloodstain extract. Most species antisera are raised against the serum of the animal, and they contain substantial titers of anti-albumin because albumin is the major protein in serum. The antisera must also be tested, at appropriate dilutions of test material, for cross-reactions with bloods of other species the examiner wants to differentiate. The cross-reactivity problem cannot be overcome altogether in the case of biologically related species.

Proper evaluation of species testing antisera with an appreciation of the antigen and antibody concentration relationships required for the assay should prevent problems that can arise from flawed technique or test conditions. If anti-human hemoglobin is being used, it too must be tested for species specificity under appropriate test conditions, at least for cross-reactivity with common household, farm, and commonly encountered wildlife animals.

The problem of true cross-reactivity is more difficult to overcome and most likely cannot be satisfactorily solved if the task is to differentiate closely related species (e.g., human and chimpanzee or wolf and coyote). Evolutionary theory predicts that closely related species will have many similarities in deoxyribonucleic acid (DNA), and therefore in protein structure. Protein sequencing and DNA sequencing have been used extensively to explore the relatedness of species. Greater similarities imply closer phylogenetic relationships. Before proteins could be sequenced, antigen-antibody reactions were used to examine the relatedness of species: This approach was based on the fact that antibodies are quite specific as to which antigens they will bind, and therefore they will cross-react only with antigens that are very similar to the antigens used to elicit them. Immunological testing to explore phylogenetic links was well underway by the time the precipitin test was described for forensic species testing: The acknowledged authority at the time was Nuttall.[16] The fact that closely related species have similar blood proteins has implications for forensic species testing, namely, that closely related species may not be distinguishable by immunological precipitin tests.

The majority of bloodstains examined in forensic laboratories are of human origin, and it is usually unnecessary to worry about the possibility that a bloodstain might have come from another primate because that possibility is not realistic in most places under most circumstances. Properly controlled and conducted tests for human blood can be considered specific if it is not possible that a nonhuman primate was the source of the blood. Similarly, tests for common farm animals, cats, dogs, and some wild game animals are specific in terms of distinguishing them from humans and from one another. In laboratories responsible for game law enforcement, the problems of distinguishing closely related species can be more of an issue. It might be important; for example, to identify wolf blood as against dog or coyote blood, and those laboratories generally turn to other methods to help them solve such problems.

Normally, the interpretation of species test results is straightforward, and test results are easy to read by the experienced professional. Positive results show that a bloodstain is from the species for which the antiserum is specific. Species tests can be done with antisera raised against the serum proteins of an animal (e.g., "anti-human" sera are generally rabbit or goat anti-human serum antibody mixtures), or they can be done with anti-hemoglobin sera. The latter are made by injecting the host animal with the hemoglobin of the animal against which antibodies are wanted. The use of anti-human hemoglobin can have the advantage of providing a confirmatory blood test and a species test in the same step, and for that reason, it is sometimes beneficial to use anti-human hemoglobin instead of "anti-human" sera. In forensic casework, however, bloodstains are not always simply dried whole blood. Shed blood can fall onto a nonabsorbent substratum and clot or partially clot before drying. In that case, the serum becomes separated from the clotted cell mass. The serum can be washed away or absorbed away by weather or some human activity. If events like these should happen, it is possible to get dried "bloodstains" that are actually composed almost entirely of cell material with very little serum. It is also possible to get serum stains that have few or no cells. "Antihuman" sera are, by definition, antibodies against serum proteins, and therefore will only react with bloodstains that have enough serum protein in them to precipitate with the antibodies. Similarly, anti-human hemoglobin sera only react with the red cell contents in a stain.

Another issue that can come up in forensic work is the fact that "anti-human" sera-that is, antisera raised against human serum proteins--do. react with some human physiological fluids, although to a lesser extent than they do with serum or blood. For example, human semen, saliva, and semen-free vaginal swab extracts all react to some extent with "anti-human" sera. The reason for this is, of course, that these fluids share some proteins with serum. This reactivity can allow "anti-human" sera to be used to test for human proteins in stains of physiological fluids if such testing is needed. It must also sometimes be considered in the interpretation of positive test results when the specimens are or maybe mixtures of blood and/or various physiological fluids.

There are a number of more involved technical modifications of precipitin tests that have been proposed or used, particularly to help in differentiating closely related species. Sensabaugh[17] has rightly noted that antisera against more rapidly evolving proteins can be expected to shown greater species specificity.

[c] Determination of Species by Typing or by DNA Analysis Methods

An additional approach to species identification can be based on typing tests, rather than on species tests, as such. Before DNA typing replaced traditional genetic-marker testing, some genetic markers had been investigated in animal species as well as in humans and shown to be human-specific. Although animal testing may not have been exhaustive in all cases, some blood group antisera designed for human blood typing yielded no reactions with animal bloods, and some human red cell isoenzymes gave patterns easily distinguishable from any known nonhuman pattern. With this knowledge, it could be argued that the results from a genetic-marker test known to be human-specific constituted a de facto species test (in addition, of course, to whatever the typing test results were). The same logic can be used if biological evidence is subjected to DNA typing, provided there is evidence to support the contention that the DNA polymorphism being typed is human-specific with the particular typing method.

There are certain known DNA sequences in many species, even closely related ones, that are species-specific.[18]

Detection of those sequences using DNA probes or by amplification using the polymerase chain reaction can be used for species testing if necessary. This kind of testing can be utilized by laboratories involved in the identification of closely related animals for game law enforcement or in the identification of exotic or rarely encountered species.

Several DNA regions have been described that are specific to humans (or at least primates).[19] The D17Z1 region is the basis of a human DNA quantitation method commonly used in forensic laboratories in the United States.[20] This method has been packaged into a commercial kit by Perkin Elmer/Roche Molecular Systems and is marketed as "QuantiBlot." In addition, some combinations of the short tandem repeat loci widely employed in forensic laboratories have been shown to yield recognizable typing results only with human specimens.[21]

§ 29.06 Physiological Fluid Identification

In the same way that bloodstains must be identified before they are typed, physiological fluid, or body fluid, stains must also be identified if possible. As noted in § 29.03[b] above, semen, saliva, and urine are most commonly seen in casework specimens. Sexual assault cases are usually, but not exclusively, the sources of body fluid evidence.

Ideally, it would be possible to rigorously identify at least the commonly encountered body fluids, if not all body fluids. The term "rigorously identify" is used to mean that testing could be done to show that a specimen was one or another body fluid to a high level of scientific certainty. Semen, like blood, can be identified with virtual certainty, but the other body fluids cannot. The tests available for fluids such as saliva, urine, and vaginal secretions are presumptive, and thus a positive identification of the body fluid cannot be made even when the test result is "positive." Methods and capabilities for typing deoxyribonucleic acid (DNA) have made possible the accurate typing of very small traces of body fluid evidence to a very high level of individuality in many cases. A laboratory worker might be virtually certain, therefore, that a biological trace originated from a particular person, but be unable to establish the nature of the biological material. This odd situation may or may not be important in a particular case. The absence of rigorous identification tests presented problems in some cases prior to the development of DNA typing methods because the traditional genetic tests provided very low discrimination potential in inclusion cases. The importance of identification versus typing tests with an evidence item has to be considered in the context of the case. Successful DNA typing on a licked envelope flap or on a swabbing from a bite mark, for example, is likely to be informative regardless of whether saliva can be rigorously identified.

It is important to recognize the limitations of body fluid identification tests so that unjustifiable conclusions axe not drawn from their results. Some of these limitations with respect to the various types of fluids are discussed below.

[a] Semen

Semen identification tests are used primarily on evidence from sexual assault cases. Typically, vaginal (and sometimes oral or anal) swabs from the sexual assault evidence collection kit, underwear, and possibly other articles of clothing are subjected to testing. A variety of other items might also be submitted for semen testing, depending on the circumstances.[22]

Semen identification tests can be divided into two categories-presumptive and confirmatory. Presumptive tests are commonly used as searching aids and as guides to selecting areas of items or items themselves for additional confirmatory testing.

[l] Presumptive Tests and Searching Aids--Ultraviolet (UV) Light, Crystal Tests, and Acid Phosphatase

[i] UV Light and Other Illumination Sources

Ultraviolet (UV) light has long been employed as an aid in searching for semen stains. Semen stains fluoresce brightly under UV light. This property can help locate semen stains on items at scenes and on clothing and other substrata, such as bed sheets and carpets. So-called "alternate light sources" and lasers are also sometimes used as aids in searching for semen stains. The principle is the same, but the alternate light and laser sources are more intense than typical UV lamps. The semen components) responsible for the fluorescence have not been characterized. Old semen stains can fail to fluoresce, and other biological stains (like saliva) may fluoresce. UV and other illumination sources are not, therefore, in any way tests for semen, but merely help in locating prospective stains for further analysis.

[ii] Crystal Tests

Over the years, there have been a number of presumptive tests for semen. Only a few have been widely used. The older ones were crystal tests, somewhat similar to the crystal tests for blood. Florence's test used iodine/iodide solution to crystallize the choline in semen out as choline iodide, while Barberio's test used picric acid to crystallize seminal spermine out as spermine picrate.[23] Chromatographic and enzymatic methods can also be used to detect choline and spermine in seminal stains.[24]

[iii] Acid Phosphatase

The most widely employed presumptive test for semen is detection (and sometimes quantitation) of the enzyme acid phosphatase. Human semen normally contains large quantities of acid phosphatase, which is contributed by the prostate gland.[25] Other common physiological fluids including serum normally have little or no acid phosphatase activity.

Tests for semen in stains using the enzyme acid phosphatase -as a marker were developed in the 1940s.[26] Kind prepared a useful review of the test in 1964,[27] and the test is still used in many laboratories for locating and screening stain specimens.

The acid phosphatase of semen may be called "AP," or "sACP," the latter to distinguish it if necessary from endogenous vaginal acid phosphatase ("vACP"). Use of AP as a semen identification marker is based on the large quantity of AP usually present in semen. AP is present in other human tissues and fluids (and in nonhuman cells), but generally in smaller amounts. Because AP is not unique to semen and because semen can have smaller amounts of AP, most experts consider this test presumptive.

There are various ways of performing an AP test, but they generally fall into two categories--qualitative and quantitative. A qualitative test is generally set up to produce a characteristic color in the presence of AP. Qualitative tests, by definition, are not designed to determine the actual quantity of AP in a specimen, but like any test, they have a lower limit of detection. Quantitative AP tests are generally spectrophotometric and are designed to measure the quantity of enzyme in the sample of specimen tested. The problem with quantitative tests in dried stains is that there is no way to know what volume of semen makes up the stain. All of the data available on AP quantity in semen is from liquid specimens, which is the only way to collect this data that makes sense. As a result, it is not possible to relate a quantity of AP found in some part of a semen stain back to the liquid specimen data in order to interpret the results of the stain test.

This problem is not limited to semen stains or to AP; it is a general problem with the interpretation of quantitative test results from dried stains.

The principal reason why so much attention was paid to the AP test is that, at one time, the only confirmatory or certain test for semen was the finding of spermatozoa (see § 29.06[a][2][i] below). The identification of semen deposited by an azoospermic male was thus a major problem in forensic cases for many years. If there were a way to make the AP test specific for semen, it could then be positively identified by that method in the absence of sperm. However; no such method was ever developed, and it no longer really matters because there is an immunological method for the identification of azoospermic semen.

Normal semen consists of seminal plasma and spermatozoa. The presence of semen in a stain can thus be identified by finding either sperm cells or a unique component of the seminal plasma. In forensic casework semen stains, there may be no spermatozoa, so that the only way to identify the stain as semen is to find a unique seminal plasma component. It is also possible in casework semen stains to find sperm cells, but very little seminal plasma, because of events that occurred between the time the semen was deposited and the time it was collected as a stain (or swabbing). To be able to identify semen in any circumstance, therefore, a method for identifying azoospermic semen is essential.

AP testing is often done on vaginal swabs or washings taken from sexual assault complainants. Over the years, quite a lot of this data has been published, and some of these studies have included quantitative AP determinations. One of the goals of these investigations was to try to find a basis for making AP a specific test for semen, as noted above, but another goal was to try to relate, AP levels in postcoital specimens to time since intercourse. Ordinarily, the AP levels in semen-free vaginal swabs are low. Immediately after coitus, the level spikes because of the semen, and then it decays as a function of time, mostly because of seminal drainage. Sensabaugh[28] synthesized the results of a series of studies of this kind and showed that AF levels in postcoital vaginal specimens followed a statistically predictable pattern. This analysis does not permit a precise calculation of time since deposit in a specific case based on the measurement of AP levels, but it does show that most of the AP activity present immediately after coitus disappears within a few hours.

To summarize, the AP test is still used for screening and locating stains. It is not a specific test for semen, and semen should not be identified solely on the basis of a positive AP test. There is a relationship in vaginal specimens between AP levels and time since semen deposit, but not one that allows an accurate estimate of time since coitus in a specific case specimen.

[2] Confirmatory Methods: Spermatozoa and p30

[i] Spermatozoa

The detection of spermatozoa is the oldest method of semen identification in stains, since the finding of sperm cells proves that semen is present: Spermatozoa in human semen were described by van Leeuwenhoek in a letter published in the Philosophical Transactions of the Royal Society of London in 1677.[29] Published methods for identifying sperm from semen stains date back to the early nineteenth century.[30] For many years, finding sperm was the only certain method for the forensic identification of semen in case specimens.

The search for sperm cells is performed with a microscope, and many different procedures may be used to recover sperm cells from stains or swabs.[31] Specimens are typically fixed onto glass microscope slides and stained with histological dyes to make the cells more easily visible. Examiners themselves usually prepare slides from stain or swab evidence items to look for sperm cells: Sometimes; medical personnel prepare slides from the swabs taken during examinations of sexual assault complainants. Those slides are placed in the sexual assault evidence collection kit for subsequent examination by forensic laboratory personnel.

Aside from its value in identifying the presence of semen in specimens, looking for sperm also helps laboratory workers judge the usefulness of evidence for DNA typing. DNA comes from cells, and sperm are the predominant cell type in semen. In some sexual assault cases that were processed before DNA typing was available, stained slides are the only remaining evidence. If the slide has sperm in adequate number, it can be recovered. and used for DNA typing.

[ii] p30 (Prostatic Antigen (PA) or γ Seminoprotein)

The identification of semen in stains and swabs in the absence of sperm was an unsolved problem in forensic work for decades. The discovery of a semen-specific protein that could be detected by immunological methods finally provided a solution. The protein that most forensic workers call "p30" was independently discovered and described by several groups. After some time, it was shown that the proteins identified by the different groups were really one and the same.

In the late 1960s, Hara and collaborators, in Japan, identified a semen-specific protein originating from the prostate gland and called it "γ-seminoprotein" (gamma-seminoprotein).[32] Sensabaugh described a similar protein called "p30" in 1978.[33] Both of these groups were interested in the protein as a forensic identification marker for semen. In 1979, Wang and collaborators described a semen (prostate)-specific protein called "prostatic antigen," or "PA."[34] The last mentioned group was interested in the protein as a potential blood serum marker for the detection of prostatic carcinoma.[35] It is now fairly clear that the three proteins identified by the three different groups of researchers are identical.[36]

Prostatic carcinoma and benign prostatic hyperplasia, which affect many men, can cause PA to be present in the blood, suggesting to clinicians the idea of using, a test for PA in blood as an early diagnostic indicator. The value of the protein in forensic science is that it allows semen to be identified in stains and swabs whether sperm is present or not. Testing for p30 is now routine in most forensic laboratories.

Tests for p30 are immunological. They can be done by passive immunodiffusion or crossed over electrophoresis, as described in § 29.05[b][1] above for species testing, or by other immunological methods. Tests for p30 can also be done by enzyme-linked immunosorbent assay (ELISA) procedures using monoclonal antibodies against p30.[37] ELISA procedures are significantly more sensitive than classical antigen-antibody detection methods, allowing smaller quantities of semen to be detected. Several membrane based immunological assays that were designed and marketed for clinical use have been evaluated for forensic semen identification testing.[38] The results of these evaluations show that these methods are valid, timesaving, and about as sensitive as ELISAs.

The p30 content of semen is distributed among different men around a mean value of approximately 0.8-0.9 mg/mL.[39] The level of p30 in semen is unaffected by vasectomy or pathological azoospermia.

[b] Saliva

Saliva cannot be rigorously identified in dried stains or swabs. The classical identification marker for saliva is the enzyme amylase, which is usually present in large quantities in saliva. However, amylase is also found in some other body fluids and in nonhuman sources. The only other human fluid that normally has significant quantities of amylase is pancreatic juice, which is secreted into the small intestine to aid in digestion. Ordinarily, pancreatic amylase is not an issue in forensic specimens, but it is present in fecal material. As a result, amylase cannot be used as a meaningful indicator of the presence of saliva if the specimen is contaminated with fecal material.

Amylase. is one of the oldest known enzymes. It catalyzes the hydrolysis of starch (a glucose polymer) and was assayed for many years on the basis of the starch-iodine reaction.[40] Iodine solutions form a characteristic deep blue color with starch, but not with starch that has been hydrolyzed. Mueller first suggested in 1928 that amylase could be used as an identification marker for saliva in stains.[41] More recently, amylase assays based on dyed starch have been developed.[42] In this method, a dye molecule is covalently attached to some of the monosaccharide units making up the undigested starch. As the "dyed" starch is subject to amylase-catalyzed hydrolysis, the colored dye-glucose molecules are released into free solution and can be quantitated spectrophotometrically. Some of the amylase assays are designed and marketed for clinical specimen testing, but can easily be adapted to amylase determination in forensic casework stains.[43]

It has been clear for some time that the salivary and pancreatic amylases in human tissues are coded for by separate, but closely linked, genes[44] and that both enzymes show some degree of variation in the human population. The salivary and pancreatic forms of the enzyme can be distinguished using electrophoresis. They can also be distinguished using specific monoclonal antibodies.[45] However, body fluids other than saliva and pancreatic juice contain smaller quantities of one or both of these forms.[46] As a result, neither method is particularly helpful in making saliva identification in stains more specific because the specimens in which saliva identification is really important might or do contain other body fluids. Moreover, neither amylase electrophoresis nor ELISAs based on monoclonal antibodies against salivary or pancreatic amylases are routine forensic laboratory methods.

The types of cases in which saliva identification has proved important have usually been sexual assaults involving oral contact. One example would be a case of an adult accused of fellating a male child in which the evidence presented is the child's underwear. Another example would be a case in which the prosecution seeks to show that a murdered woman was sexually assaulted by cunnilingus. In the former type of case; the evidence might also have traces or stains of urine or perspiration on it, and both those fluids have some amylase in them. The latter type of case usually arises if the crime appears to have involved sexual assault, but no semen is found on the vaginal swabs, and the swabs are then tested for amylase. The problem in these types of cases arises from the interpretation of a positive amylase test result. It is clear that saliva cannot be identified on the basis of an amylase test. Like acid phosphatase, discussed in § 29.06[a][1][iii] above in connection with semen identification, amylase is not unique to saliva, and quantitative amylase tests in dried stains or swabs suffer from the same interpretation problems as acid phosphatase tests, i.e., quantities of amylase measured in stains cannot be related to knowledge of quantities in liquid saliva because the volume of saliva that formed the stain is unknown. In addition, amylase is present to some extent in vaginal material, urine, perspiration and other body fluids. There is also evidence that postmortem semen-free, saliva-free vaginal swabs can have significant amylase activity, especially if the body has started to putrefy.[47]

Presumably the result of putrefaction and/or postmortem autolysis (because semen-free, saliva-free vaginal swabs from living women have relatively small quantities of amylase), this situation makes interpretation of amylase levels on vaginal swabs from deceased women almost impossible.

There are salivary proteins other than amylase that could undoubtedly be used as the basis for a rigorous forensic identification test,[48] but the research and validation work with those proteins has not been done. It may be that a rigorous saliva identification test is not as important as it was before the availability of DNA typing. In either type of case described in the examples above, DNA typing would provide powerful evidence, since the finding of a suspect's DNA type in the stains on a child's underwear or on a postmortem vaginal swab probably could not be readily or innocently explained. With the availability of such DNA evidence, it might not matter much that saliva, assuming it was the body fluid involved, could not be identified with certainty.

[c] Urine

Urine contains relatively large quantities of creatinine and urea, and identification tests for urine in stains have generally been based on one or both of those compounds. Jaffe showed in 1886 that creatinine forms a characteristic precipitate with picric acid,[49] and this method, along with other methods for demonstrating the presence of creatinine, have been used to identify urine in stains. Various methods for the detection of urea for urine stain identification have also been described.[50] Weinke et al. suggested using thin-layer chromatography to detect both urea and creatinine in an effort to make the test more specific for urine, and others have suggested detecting additional components of urine on TLC plates for the same purpose.[51]

The concentration of any compound in urine depends on body weight and on the quantity of urine produced. A normal person weighing about 150 pounds who excretes 1 L of urine in a day has 1.4-3.5 g urea and 105-210 mg creatinine per 100 mL urine on average. Clinical chemists have long measured creatinine levels in urine as one index of normal health, and forensic toxicologists use creatinine levels in urine specimens submitted for forensic urine drug testing as an indicator of whether the specimen may have been purposely diluted.

Urea and creatinine levels in urine are well above those in other, common body fluids in normal people. The question is whether detection of these compounds in relatively high amounts constitutes a specific test for urine. The consensus answer is that it probably does not, and most authorities regard these tests as presumptive, even when both compounds are detected. Extensive validation work to show that either or both compounds in some defined quantity constitute a specific urine identification test has never been done. In any event, it is unlikely that the outcome of a forensic case would ever depend on the rigorous identification of urine.

[d] Other Physiological Fluids

In some forensic cases, it can be useful to be able to identify vaginal material, perspiration, gastric fluid, or fecal material. Attempts to identify each of these fluids present certain problems, however.

The material on a semen-free, blood-free vaginal swab is sometimes called vaginal "fluid" or "secretions," but, in fact, it is neither. It is a mixture of exfoliated epithelial cells, mucous, transudate, and bacteria that inhabit the normal vagina. The epithelial cells are indistinguishable from the epithelial cells of other mucous membrane linings of the body, and there are no known proteins or small molecules that are specifically vaginal. As a result, there are no "tests" for the identification of this material.

Occasionally, sexual assault cases arise in which the woman was not subjected to intercourse or oral assault, and thus a vaginal swab shows no semen or saliva evidence. If the perpetrator used his fingers or an object to penetrate the woman's vaginal vault, however, an. "identification" of vaginal material on an object or on clothing, etc., would help corroborate her account of the events. Although no such identification is possible, DNA typing on such evidence could show to a high degree of certainty that the material originated from the victim.

There are no specific tests for perspiration, and the identification of perspiration rarely, if ever, arises in casework. Before DNA typing, it would sometimes have been useful to be able to identify perspiration stains because perspiration could be mixed with other fluids, like semen or blood, and perspiration could contribute blood group substances to those mixtures.

Gastric fluid can be identified because it contains the unusual enzyme pepsin, a proteolytic enzyme with a pH optimum of around two. This property means that it is maximally active in extremely strong acid solutions (because the stomach secretes hydrochloric acid to aid in digestion). Although gastric fluid identification is by no means routine in forensic laboratories, a procedure has been described for it.[52]

There are some reports in the older literature about methods for identifying fecal material, although the issue comes up only rarely in casework. It is fair to say that the research to construct and validate a good identification method have never been done, even though there are probably detectable compounds that could be used for this purpose, alone or in combinations. Since fecal material contains a variety of undigested or partially digested foodstuff materials, some investigators have suggested that specimens can .be compared for common origin. Harmless parasites and their eggs have sometimes been used as part of these comparisons.[53]

§ 29.07 Individualization of Blood and Body Fluid Evidence,

[a] DNA Typing

Forensic typing of deoxyribonucleic acid (DNA) began around 1985, and DNA typing continues to develop at an astonishing pace. As the twentieth century draws to a close, laboratories are implementing what could be called the third generation of DNA typing technology.

[b] Review of Traditional Genetic Markers

There are five major classes of traditional genetic markers (individualizing genetic characteristics):

(1) Blood group antigens;
(2) Red cell isoenzymes;
(3) Serum or plasma proteins;
(4) Certain hemoglobin variants; and
(5) The HLA system antigens.

Each of the first three classes has a number of different "systems," and many of these markers have proved useful in bloodstain analysis. A "system" refers to a protein, enzyme, or antigen that shows multiple types among individuals attributable to multiple genes at a single genetic locus (location within the entire DNA). The traditional genetic-marker systems used in forensic biological evidence analysis are summarized in Table 29-4 below.

The traditional systems for typing biological evidence are called "genetic markers" because they are inherited in a consistent, predictable way and because the types vary in populations. The genes responsible for these systems behave according to well-understood rules in individual mating, in families and pedigrees, and in populations. As a result, the types can be used to distinguish between individuals in populations.

When genetic-marker types are found in evidence items, they are compared with the types of one or more people to determine whether they match. If a person's genetic-marker types do not match the types found in the evidence, the person is excluded. Exclusions are absolute proof that the person did not deposit the evidence. A finding that the types do match does not conclusively indicate that the evidence came from that person. Rather, such a result means that the person is included in a segment of the total population of persons who might have deposited the bloodstain because everyone in that segment of the population has that same set of types. In other words, traditional genetic markers can partially, but not completely, individualize biological evidence.

A person's inherited characteristics are, of course, the result of differences in DNA sequences. At one time, those variations could not be detected easily at the DNA level. When a gene has several different forms in 'a population; and thus expresses differently in different individuals, its locus is said to be "polymorphic." Genetic polymorphism is fairly common in human populations and is the basis of all forensic genetic typing, both by traditional methods and by DNA.

Traditional genetic markers for the individualization of biological evidence have been entirely displaced by DNA typing. Not only are some kinds of DNA typing far more individualizing than any one or combination of traditional markers, DNA is significantly more stable in biological evidence than were the traditional genetic markers.

There is still occasional interest in the traditional marker systems, usually because older cases sometimes resurface in which there is insufficient material for DNA typing. Traditional genetic-marker systems have been extensively reviewed elsewhere,[54] and thus are described only briefly below.

[1] Blood Groups

[i] Bloodstains

The major blood group systems are ABO; Rh, MNSs, Kell, Duffy, and Kidd. Each system is controlled by its own 'genetic locus. A person's blood group in any system is determined by the presence or absence of blood group antigens on the surface of the red cell. Specific antibodies are used to test a person's red blood cells for the presence of any one of these antigens. A series of specific antibodies can be used to determine a person's red cell antigen types in the different blood group systems. When blood dries, the red cells lyse (rupture), and thus a bloodstain does not have any intact red cells. For this reason, bloodstains cannot be typed like whole blood because there are no red cells to work with, and indirect methods, described briefly below, must be employed.

Blood groups were the first category of human genetic marker. The ABO blood group system, first described in 1901 by Landsteiner,[55] is by far the most commonly typed system in bloodstain analysis. The ABO system consists of four basic types: A; B; AB; and O. A person's ABO type is determined by which antigens are present on the red cells.

The cells are typed with anti-A, anti-B, and a third reagent called anti-H (which reacts with type O red cells). The ABO system has another property that makes it peculiar among the blood group systems-an individual's serum contains antibodies corresponding to the antigens that he or she lacks. Thus, type A people have anti-B, type B people have anti-A, type O people have both, and type AB people have neither. As a result, blood can be typed for ABO using either red cells or serum. ABO is the only blood group system in which people have "naturally occurring" blood group antibodies of this kind.

In his original description of the ABO blood group system, Landsteiner noted that ABO antibodies were still detectable in a two-week-old bloodstain on linen. People interested in typing bloodstains subsequently tried to develop typing methods based on the detection of the serum antibodies. Lattes was the first, beginning in1913, and he did considerable research and casework in this area.[56] Bloodstain ABO typing by detection of the serum antibodies is called the "Lattes procedure."

Over time, forensic workers developed ABO typing methods based on detecting the blood group antigens. The first method used was absorptioninhibition,[57] but another more sensitive method, called "absorption-elution,"[58] eventually became the standard procedure.

Because the ABO antigens are considerably more stable than the antibodies in dried stains, many laboratories did not use the Lattes procedure in routine work. For various reasons, there was general agreement that a bloodstain could not be conclusively typed for ABO using either the Lattes method or the elution method. Conclusive typing would require that both tests be done. Many laboratories did not use the Lattes test because it only gave reliable results in fresh bloodstains, and many of the bloodstains being examined in forensic laboratories were already too old to expect Lattes results. As a consequence, laboratories reported the ABO antigens they detected in bloodstains, but would not conclusively assign blood types.

The absorption-elution technique can also be used to type other blood group system antigens.[59] For a time, quite a few laboratories typed MN in addition to ABO. Biochemical studies in the 1970s showed that there was an intrinsic problem with the reliability of MN typing in bloodstains, however, and the system was dropped. Very few laboratories in the United States typed the other blood group antigens in bloodstains, but such typing was somewhat more common in Europe and elsewhere.

[ii] Body Fluid Evidence

The ABO antigens were typed routinely in forensic body fluid specimens for many years, and the system is expressed somewhat differently in body fluids. Most of the genetic-marker systems found in blood are not expressed in body fluids, although ABO blood group antigens, a few isoenzymes, and perhaps immunoglobulin allotypes are. Thus, until DNA typing emerged, there were few useful genetic markers for semen in sexual assault cases.[60]

In the 1930s, it was found that ABO blood group substances could be present in large quantity in body fluids,[61] and that this characteristic was genetically controlled. About 75% of most population groups were found to have ABO in body fluids and were called "secretors. " The remainder, who had little or no such ABO substances in body fluids, were called "nonsecretors." The ABO substances found in secretor body fluids corresponded to the individual's blood group, except that secretors of all the blood groups were found to have substance H (see Table 29-3).

Forensic scientists quickly took advantage of this finding[62] because it provided a method for including or excluding possible semen depositors in sexual assault cases. The method, primarily used to detect A, B, and H in body fluids was absorption-inhibition, and there are several variations of that test.[63] Typically, a person's saliva was tested to determine secretor status. Sexual assault evidence-almost always a mixture of fluids from the woman and the offender could be tested to determine the ABO antigens present. To interpret the findings from the evidence, the blood group and secretor status of the two people had to be determined. A suspect could then ordinarily be included or excluded as a possible depositor.

Table 29-3. ABO Group Substances in Body Fluids of Secretors

Blood Group	ABO Blood Group Substances in Body Fluids
O	H
A	A + H
B	B + H
AB	A + B + H

[2] Isoenzymes

Enzymes are the body's catalysts; they speed up chemical reactions, which would otherwise be too slow to maintain life. Red cells, tissues, and body fluids contain many enzymes. Some of the enzymes in human red cells and tissues have been found to be controlled by polymorphic gene loci, so that different individuals will have different forms of the enzyme. The different forms can be detected using appropriate methods, thereby giving genetic information about the person. Enzymes controlled by polymorphic genetic loci, and thereby exhibiting multiple genetically controlled forms, are called "isoenzymes."

The most important red cell isoenzyme systems used in forensic serology were as follows: phosphoglucomutase (PGM); acid phosphatase (ACP or EAP); adenylate kinase (AK); adenosine deaminase (ADA); esterase D (ESD); glyoxalase I (GLO); carbonic anhydrase II (CA II); glucose-6-phosphate dehydrogenase (Gd or G6PD); 6phosphogluconate dehydrogenase (PGD); glutamic-pyruvic transaminase (GPT); and peptidase A (PEPA). Not all of them were equally useful. Their characteristics as genetic markers are summarized in Table 29-4.

Isoenzyme typing is done by a procedure called electrophoresis, a process for separating different protein molecules (which the isoenzymes are) in an electric field because of differences in their net charge. In practice, electrophoresis is carried out on different types of gels (made of starch, agarose, polyacrylamide, etc.). These different gels are prepared on glass or plastic carriers, which can be placed into electrophoresis chambers. The specimens to be analyzed are applied to the gel, and the electrophoresis chamber allows an electric current to be applied throughout the gel. Positively charged proteins will migrate toward the negative electrical pole, while negatively charged proteins will migrate toward the positive. If the isoenzymes of interest are differently charged under the test conditions, they can be separated in this way, and detected later, after the electrophoresis has finished. A related protein separation procedure, which works on a different principle, is called isoelectric focusing, and it has also been used to separate some of the proteins and enzymes that are important in bloodstain analysis.

Table 29-4. Summary of Traditional Genetic-Marker Systems

System	Number of Types*	Discrimination Index	
		Caucasian	African-American
Blood Groups			
ABO	4	0.62	0.64
Rh	7	0.79	0.69
MNSs	9	0.83	0.78
Kell	2	0.15	0.04
Duffy	3 or 4	0.62	0.56
Kidd	3	0.61	0.55
Isoenzymes			
PGM	3 (or 10)	0.52 (0.75)	0.47 (or 0.73)
ACP	5	0.66	0.54
AK	2	0.13	0.04
ADA	2	0.18	0.10
ESD	3	0.35	0.27
GLO	3	0.61	0.62
CA II	1 or 3	- -	0.33
PGD	2	0.09	0.13
GPT	3	0.63	0.48
PEPA	1 or 3	- -	0.21
Serum Groups			
Hp	3	0.62	0.72
Gc	3 (or 6)	0.57 (or 0.75)	0.33 (or 0.67)
Tf	2	0.04	0.15
Gm / Km	many	- -	- -
Hemoglobin Variants	3	- -	0.04

* The different numbers shown reflect that there may be different numbers of types in different ethnic / racial populations or that isoelectric focusing methods revealed additional types, thus increasing the Discrimination Index.

Most isoenzyme systems were originally discovered using electrophoresis. In some cases, when the systems were examined using isoelectric focusing, additional genetically controlled forms were discovered. In those cases, the additional or more complete sets of forms were called- "subtypes" to distinguish them from the original "types" seen by electrophoresis. Although the choice of that term was unfortunate, it was widely accepted and used.

[3] Serum Groups

Some of the proteins in serum have several forms, like the isoenzymes described above. They are not enzymes, however, and have to be detected differently. The most important proteins used in forensic serology were the immunoglobulin markers (Gm and Km), haptoglobin (Hp), group specific component (Gc), transferrin (Tf) and protease inhibitor (Pi). Except for Gm and Km, which were typed serologically (somewhat like red cell antigens), the other systems were all typed by electrophoresis or isoelectric focusing techniques.

The genetic properties of individual systems are summarized in Table 29-4 above.

[4] Hemoglobin Variants

Hemoglobin can show inherited variations, and a few of the variant hemoglobins occur frequently enough in populations to have been useful bloodstain genetic markers. Hemoglobin variants were determined by electrophoresis or isoelectric focusing.

[5] HLA System

The. HLA system was the most complex antigen system discovered. HLA antigens constitute the histocompatibility complex, and mismatches in HLA types are the reason for tissue transplant rejection. HLA antigens occur in tissues and on the white blood cells, so that lymphocytes were commonly used for HLA typing. The typing was done by serological procedures somewhat similar to those used to type red cell antigens, but HLA typing procedures were more complex and more difficult. Good methods for typing HLA in dried blood were never developed, but the system was widely used in-disputed parentage and disputed paternity testing until it was displaced by DNA typing methods.

[c] Principles of Biological Evidence Individualization

Individualization of a biological evidence specimen involves comparison of the genetic-marker types or, under modern methods, DNA types in the specimen with those of people who might have been depositors. A person whose types do not match the evidence types is excluded. In inclusion cases in which the specimen types do match the types found in some identified person, genetic-marker type frequencies in populations are used to calculate the fraction of the population that can be included as possible depositors of the evidence (along with the person in the case). Thousands of population studies done over many decades provided the data used for this purpose with the traditional marker systems.[64]

Population data for systems in populations are subjected to statistical tests for Hardy-Weinberg equilibrium (HWE) and forr independence. HWE allows the calculation of expected type frequencies in a population within reasonable confidence intervals from the frequencies of a population sample (e.g., a few hundred unrelated individuals). Independence must be shown to allow use of the so-called product rule -- multiplying together the individual system (locus) type frequencies to get the overall genetic profile frequency.

Profile frequencies decrease as more systems are included in a profile. With traditional genetic-marker systems, no one system was highly polymorphic. As a result, laboratories tried to add more and more systems to their routine profiling to better individualize evidence. Even under the best circumstances, however, evidence could never be individualized to a degree even approaching that obtained by DNA typing methods.

It is clear that genetic-marker type frequencies vary in different human populations, such as Caucasians, African-Americans, and Chinese. In the United States, data has generally been collected for Caucasians, African-Americans, and separately for "Hispanic" populations, and sometimes for Chinese, Japanese, or Native American people. As a rule, laboratories provide genetic profile frequencies for all the populations for which data are available, since the ethnic or racial origin of the actual evidence depositor is not known.

A parameter referred to as the Discrimination Index (DI), which can be calculated from population genetic data for genetic-marker systems, is actually the probability that two randomly selected, unrelated individuals in a population will have different types, i.e., will be discriminated.[65] The DI, shown in Table 29-4 above, provides a measure of the relative usefulness of a system for individualization.

[d] Other Characteristics of Blood-Sex of Origin, Presence of Antibodies, and Presence of Drugs

Before DNA typing methods were available, there was some research on the possibility of using other detectable characteristics-including sex of origin, presence of various antibodies, and presence of drugs-as tools for bloodstain individualization.[66] Bloodstains could, in theory, be tested for any, of three characteristics to determine whether they were of male or female origin: Barn bodies; F bodies; or sex hormone levels. Barr bodies, which were originally described by Barr and Bertram,[67] are characteristic of the nuclei of mammalian female cells. For bloodstains, the fluorescent Y chromosome (F body) technique was the most promising. Sex hormone quantitation by radioimmunoassay techniques was also successfully applied to the problem. Although these techniques appeared to be reliable in skilled and experienced hands, they were never in widespread use in the United States. The commonly used modern DNA typing techniques routinely provide sex of origin results. The determination of syphilis antibody, anti-parasitic antibodies, antibodies present as the result of infection or allergy, hepatitis B antigen, and various drugs in bloodstains were all investigated as other potential means of comparing bloodstains with possible depositors prior to the availability of DNA typing.

§ 29.08 Evidence Collection and Preservation

[a] Blood Evidence,

No single prescription for handling evidence can be given that would apply to every case. Evidence collection requires judgment and training, and the techniques to be used should be decided in consultation with laboratory personnel. Few considerations are as important as, preserving the integrity of a scene. Every feature of a scene should be recorded by photography and by sketches that include good measurements. More cases are made difficult by activities at the scene than by any analytical procedure in the laboratory. Evidence collected at the scene must, of course, be carefully labeled to preserve the chain of custody.

Dried blood specimens are best packaged in paper containers, and never in airtight containers where bacterial or mildew contamination might develop. Bloodstains should be thoroughly dry before packaging. Bloodstain patterns are often critical to reconstructing events, and careful records should be made of patterns before they are destroyed by collection of the blood. If any field testing is to be done, it should be carried out by specially trained investigators or supervised by someone from the laboratory.

[b] Sexual Assault Case Evidence

Sexual assault case evidence can, but does not always, consist of three separate groups of items: evidence from the victim and the victim's person; evidence from the scene; and evidence from the suspect. Like blood evidence, as discussed in § 29.08[a] above, body fluid stains and swabs collected in sexual assault cases should be thoroughly dry before packaging.

Evidence from the victim and the victim's person is generally collected in a clinical setting by physicians or nurses: Clothing is usually collected, and in many jurisdictions, a sexual assault evidence collection kit is used to collect evidence from the victim's person. The evidence collection kits vary from place to place, although there has been a significant effort to standardize them. In some states, there is a standard kit, and a legislatively mandated coordinating committee is responsible for specifying the kit's components. Where a kit is used, provision is generally made for collecting articles of clothing and debris and other material that may be adhering to the clothing. In addition, dried stains on the victim's body and vaginal, anal, and oral swabs and slides are collected. Some kits make provision for collecting "foreign" and known hairs, and fingernail clippings. A known blood specimen (or a buccal scraping) is also collected. The slides are used to look for sperm, and the swabs are generally used for semen identification tests and deoxyribonucleic acid (DNA) typing. The "best evidence" in a given case depends on what happened and how long the victim waited to come forward. The more time that elapses between the sexual assault and the physical examination, the less likelihood that good semen evidence on vaginal or other swabs will be found. Panties that were worn following the assault can provide good semen evidence. In some cases, the best semen evidence might be found in stains on clothing.

The evidence collected from the scene should include any items in which biological evidence might be found. Scenes should also be processed for other physical evidence.

Some jurisdictions provide "suspect" evidence collection kits. These make provision for collection of clothing, a known blood specimen or stain or buccal scraping, and penile swabs. Some kits have been designed to be used for victims or suspects by following separate instructions and using the kit's contents for different purposes.

Condoms are sometimes collected as evidence in sexual assault cases. A condom can provide very good evidence because there is semen on the inside and vaginal epithelial cells on the outside.

The critical feature in handling sexual assault cases is cooperation by all the parties involved: investigators; examining clinicians; and laboratory personnel. Errors made in evidence collection in connection with the physical examination are often irreversible. As noted above, some jurisdictions have spontaneously, or following legislative mandate, established coordinating committees to set guidelines for medical examinations and specify the makeup of evidence collection kits. These committees usually include representation from laboratory personnel, law enforcement; clinicians, prosecutors, and victim services agency personnel. The development of forensic nursing as a specialty has helped in the process of sexual assault evidence collection in many jurisdictions. Some jurisdictions also have Sexual Assault Response Teams (SARTs) that swing into action when there is a victim to be seen. Besides evidence collection, there are medical and health matters that must be properly handled, and follow-up is generally indicated.

Drug-assisted sexual assault (i.e., sexual assault involving "date-rape drugs") is a growing problem as of this writing. The full extent of the problem is not completely known and is not easy to establish by normal scientific or epidemiological methods. From a forensic science viewpoint, toxicological analysis is indicated in such a case along with the usual biological evidence identification and DNA typing, and specimens must be separately collected for that purpose. Blood and urine are the typical toxicological specimens, but if too much time has elapsed between the assault and the examination, they may no longer contain detectable quantities, of the drug. To deal with those situations, there is active, ongoing research into the possibility of using hair to detect the drug even weeks or months after administration.

One of the problems with some "date rape drug" cases is that some of the drugs employed for the purpose of the assault are also abused in recreational use, thus providing a suspect with an "innocent" explanation for the toxicological findings. In addition, victims may have little or no recollection of the events and may not be able to provide much information about what happened.

Sexual assault evidence collection, except for the possible collection of specimens for toxicological analysis, is focused on gathering the best evidence for DNA typing. Not only is DNA typing a powerful tool for individualizing such evidence, but DNA profiles can also be compared with those in offender data banks in many states and nationally.

The literature on sexual assault and evidence collection is vast. However, there are no summaries or reviews readily available.

[c] Saliva Traces from Bite Marks

In the pre-DNA era, saliva was collected from people for routine secretor status determination, but this is no longer necessary. It may be necessary on occasion to collect saliva from a body around the area of a bite mark. This should be done in cooperation with a qualified forensic odontologist, so that the bite mark characteristics are not affected.[68] The area can generally be swabbed to collect saliva traces, but very small amounts of swabbing material should be used, since the amount of saliva is likely to be small. It is also necessary in these situations to collect "control" swabs from areas of the body near the bite mark to show that the DNA profile assumed to be from the saliva is not a contaminant.

§ 29.09 Continuing Importance of Forensic Serology Activities in Era of DNA Typing

In 1904, Dr. Florence, a well-known medicolegal figure of his time (and inventor of the "Florence test" for semen identification) sarcastically commented on the possibility of "individualizing" a bloodstain specimen: "It is no longer a matter of distinguishing rabbit blood from human blood, but rather of saying that this stain was made by the blood of Pierre, and not by that of Paul or Francois."[69] A quarter century later, Dr. Landsteiner, who was awarded the Nobel Prize for Physiology or Medicine in 1930 for the discovery of human blood groups, said in his laureate address: "These findings justify the assertion that very numerous individual blood differences exist in man : . . and that there are certainly other differences which could not yet be detected. Whether each individual blood really has a character of its own, or how often there is complete correspondence, we cannot yet say."[70]

As the curtain falls on the twentieth century, bloodstain and biological evidence individualization is essentially a reality through deoxyribonucleic `acid (DNA) typing techniques and technologies. Traditional genetic-marker testing has been abandoned and takes its place as a chapter in the history of forensic biological evidence analysis.

Notwithstanding the DNA typing revolution, some activities long associated with "forensic serology" remain important and continue to be a vital part of forensic biological evidence analysis. Blood and physiological fluid stains and traces still require identification. More importantly, those aspects of "forensic serology" most characteristic of its association with criminalistics remain critical if biological evidence analysis is going to help unravel a case. Recognizing the crucial physical evidence in a given case, using experience and judgment to select the most important and informative specimens for typing in terms of the case, and interpreting stain patterns are essential criminalistics skills that cannot be replaced by any DNA typing technique.

References / Notes

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