FORENSIC SEROLOGY

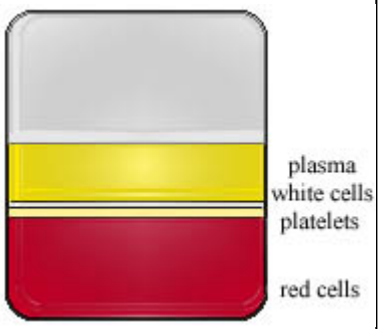
Determination of the type and characteristics of blood, blood testing, bloodstain examination, and preparation of testimony or presentations at trial are the main job functions of a forensic serologist, who also analyzes semen, saliva, other body fluids and may or may not be involved with DNA typing. It must be recognized, however, that in many crime labs, there may be no clear distinction between job title and job function. A particular laboratory may not have a serologist on staff, their functions being performed by a criminalist, a biochemist, a forensic biologist, or other technician. Such personnel would normally possess a Bachelor's or Master's degree, while a chief serologist would possess an M.D. or Ph.D. It's rare to find chief serologists, and the Bachelor's degree seems common. A few states have laws which make serological examinations admissible by statute without the necessity for testimony by an expert, the purpose of which is to insulate and protect their crime lab technicians. Other states rely upon their Chief Medical Examiner's office, forensic pathologists, or board-certified toxicologists. Professors of biochemistry, hematology, and immunology are often "borrowed" as experts by both prosecution and defense.

In certain specialized areas involving bloodstain examination (such as blood spatter analysis), courts will ordinarily qualify someone as an expert who has no formal education but specialized training and has conducted a sufficient number of examinations and accumulated enough reference patterns to be able to demonstrate the basis of their opinion. These kinds of experts are usually law enforcement personnel, and their testimony is most frequently found in those states which have modified *Frye* or embraced *Coppolino*. Further, blood and bloodstain evidence is such an integral part of most crime scenes that a police investigator/bloodstain specialist might be found, in some jurisdictions, testifying on the ultimate issue, even though this usurps the province of the jury. Federal Rule of Evidence 704 allows this to some degree. The *Daubert* impact has brought conditions more in line with Federal Rule of Evidence 702 than with a statistical showing of validity and reliability. Probability estimates are frequently used in blood testimony.

Blood is the most common, well-known, and perhaps most important evidence in the world of criminal justice today. There's no substitute for it, whether for medical or forensic purposes. Its presence always links suspect and victim to one another and the scene of violence. Bloodstain patterns tell a lot about position and movement during the crime, who struck whom first, in what manner, and how many times. This destroys most alibi and self-defense arguments for crime, and at the very least, trips most suspects up in their explanation of what happened. Over the years, criminals have tried many ingenious ways to hide, clean up, and remove blood evidence, but it's an area where criminal justice technology has always stayed

one step ahead of them.

Blood is a slightly alkaline fluid made up of water, cells, enzymes, proteins, and inorganic substances that circulate throughout the vascular system carrying nourishment and transporting oxygen and waste. The most fluid portion of blood consists of *plasma*, which is mostly water, and *serum*, which is yellowish and contains *white cells* and platelets. The most non-fluid portion of blood consists of *red cells* which outnumber white cells by five hundred to one. While medical scientists are more interested in white cells, forensic scientists are more interested in red cells and secondly with serum. With serum, the analyst can determine the freshness of a blood sample because serum clots several minutes after exposure to air (a centrifuge is necessary to separate clotted material from the rest of serum). In serum are also found *antibodies*, which have important forensic implications. With red cells, the analyst looks for smaller substances residing on their surfaces, such as *antigens*, which have important forensic implications. One might even say that *forensic serology is all about antigens and antibodies*, but that is the domain of immunology.



In forensic law, blood has always been considered class evidence, but the potential exists for individualized blood typing, and even today, forensic serologists can provide testimony with some strong probability estimates linking a single individual, and that individual only, to a bloodstain. Consider that identical twins may have the same DNA profile but completely different antibody profiles, and you begin to see how promising the field of forensic serology really is.

BLOOD TYPING

The typing of blood, with what is now called the A-B-O system, was discovered in 1901. A few years later, starting around 1937, a series of antigen-antibody reactions were discovered in blood, the most common ones being ABH, MN, Rh, and Gm (over 100 antigens exist). Most people are only familiar with the Rh factor, which is technically the D antigen. There are more than 256 antigens, and 23 blood group systems based on association with these antigens.

As you can see from the graphic of this microscopic image, there are a lot of components surrounding blood cells. *Antigens* are chemical structures attached to the surfaces of red blood cells. *Antibodies* are proteins floating in blood fluid (the serum,

specifically, and platelets, associated with clotting), and exist because people have allergies or may have come in contact with a common disease (TB, smallpox, and hepatitis are common antibodies). Blood may also contain HIV antibodies, syphilis, and cholesterol. The most common problem (hematological condition) with blood is an iron deficiency. Iron is essential for the production of hemoglobin, the red pigment in cells, and iron also makes an excellent transport vehicle for nutrients. Anemia is a related condition involving a deficiency in the number of red blood cells.

A basic principle of serology is that for every antigen, there exists a specific antibody. In fact, ALL BLOOD GROUPS ARE DEFINED BY THE ANTIGENS ON THEIR RED BLOOD CELLS AND ANTIBODIES IN THEIR SERUM.

Blood type:	*Antigens on red cells:*	*Antibodies in serum:*
A	**A**	**Anti-B**
B	**B**	**Anti-A**
AB	**AB**	**Neither anti-A or anti-B**
O	**Neither A nor B**	**Both anti-A and anti-B**

For routine blood typing, all you need are two *antiserums*: anti-A and anti-B, both easily available commercially. By dripping a droplet of these antiserums in samples of blood, you see which samples maintain a normal appearance (at about 200x magnification) and which samples become clotted, or agglutinated. Blood of type A will be agglutinated by anti-A serum; blood of type B will be agglutinated by anti-B serum; AB blood by both; and O blood by neither. You are essentially determining blood type by injecting the worst possible poison into someone's blood sample to see what happens. Also, despite some racial and geographical variation, blood types are normally distributed in a population as follows:

O	A	B	AB
43-45%	**40-42%**	**10-12%**	**3-5%**
O+ 39% O- 6%	**A+ 35% A- 5%**	**B+ 8% B- 2%**	**AB+ 4% AB- 1%**

The "O" type is most common among indigenous people (like Aborigines and Native Americans) and Latin Americans. The "A" type is most common among Caucasians and those of European descent. The "B" type is most common among African-Americans and certain Asians (e.g. Thai). The "AB" type is most common

among the Japanese and certain Asians (e.g. Chinese). An interesting phenomenon is that Middle Easterners are somewhat likely to have nucleated red blood cells, whereas normally, red blood cells contain no nucleus. Men generally have more red blood cells than women. Red blood cells are originally formed from stem cells, and stem cells are found in bone marrow, the ribs, breastbone, pelvis, and vertebrae, but red cell production is controlled by a hormone released by the kidney, which in turn, instructs the bones to release more red blood cells. Rare blood types exist in addition to the basic ABO system.

A far more useful breakdown involves the Rh (Rhesus disease) factor. If a person has a positive Rh factor, this means that their blood contains a protein that is also found in Rhesus monkeys. Most people (about 85%) have a positive Rh factor, and doctors are trained to monitor closely any woman who is Rh negative and becomes pregnant. The Rh system is actually much more complicated than the ABO system because there are about 30 combinations possible, but for the sake of simplicity, Rh is usually expressed as either positive or negative. The Rh factor, like other antigens, is found on the covering of red blood cells. It's common for a forensic scientist to take the percentage distribution of the Rh component, which is expressed as plus or minus, to present some of the blood groups in terms of odds-ratios:

O+	**1 in 3 persons**
O-	**1 in 15 persons**
A+	**1 in 3 persons**
A-	**1 in 16 persons**
B+	**1 in 12 persons**
B-	**1 in 67 persons**
AB+	**1 in 29 persons**
AB-	**1 in 167 persons**

Subgrouping is also possible under the ABO system. Various extracts can be obtained from plants and seeds to create antiserums that clot type O blood, for example, somewhat selectively. Most major blood groups have at least two major subgroups; O1, O2, A1, A2, etc. The most commonly used types of antiserums used for this purpose are called *lectins*.

The possibility of individualized blood types is based on the typing of proteins and enzymes. Forensic serologists almost always do this level of typing. Blood proteins and enzymes have the characteristic of being *polymorphisms* or *iso-enzymes*, which means they exist in several forms and variants, so each one of them have subtypes. Most people are familiar with at least one common polymorphism in blood: Hb, which causes sickle-cell anemia. The following are some common polymorphisms:

PGM 2-1	**phosphoglucomutase**
EAP	**erythrocyte acid phosphatase**
EsD	**esterase D**
AK	**adenyl kinase**
ADA	**adenoisine deaminase**
GPT	**glutamic pyruvate transaminase**
6-PGD	**6-phosphogluconate dehydrogenase**
G-6-PD	**glucose-6-phosphate dehydrogenase**
Tf	**transferrin**

Each of these protein and enzyme variants, as well as all blood subtypes, have known distributions in a population. It's therefore a simple matter to calculate probability estimates that border on individualized blood typing. (*Let's do the math*) Suppose you had a crime scene sample and a suspect which both were characterized by type A blood (42%), basic subtype A2 (25%), protein AK (15%) and enzyme PGM 2 (6%). The probability of finding two people in the population with this exact type would be less than 0.000945 (.42 x .25 x .15 x .06). The closer you come to producing a number out sixty decimal places, the more you've achieved saying there's no one else on Earth who could have committed the crime. Juries are usually impressed, however, by numbers out four, five or six decimal places, and the defense is put in the awful position of having to put a mathematician on the stand to lecture them about how many decimal places should be impressive.

BLOODSTAIN CHARACTERIZATION

The science of bloodstain analysis somewhat traditionally follows certain steps which serve to adequately describe the various tests conducted. Those steps are:

1. **Is the sample blood?**
2. **Is the sample animal blood?**
3. **If animal blood, from what species?**
4. **If human blood, what type?**
5. **Can the sex, age, and race of the source of blood be determined?**

To answer Question 1, forensic scientists use color or crystalline tests. It used to be that courts trusted police investigators who said they knew blood when they saw it, but that was before *Miller v. Pate* (1967) where someone got stumped on a cheap lawyer trick with red paint on clothes. The *benzidine test* was popular for awhile until it was discovered to be a known carcinogen, and was replaced by the *Kastle-Meyer test*, which used the chemical phenolphthalein. When it comes in contact with hemoglobin (and sometimes potato and horseradish), phenolphthalein releases

peroxidase enzymes that cause a bright pink color to form. To detect invisible blood stains, the *luminol test* is used, which is a chemical sprayed on carpets and furniture which reveals a slight phosphorescent light in the dark where bloodstains (and certain other stains) are present. Long-dried blood has a tendency to crystallize, or can be made to crystallize with various saline-acid mixtures, and the names of various crystal tests are the *Teichman test*, the *Takayama test*, and *Wagenhaar test*. The generic term for any way of determining if something is blood or not is called a presumptive test.

To answer Questions 2 and 3, forensic scientists use antiserum or gel tests, and you may ask why it's important to test for animal blood. The answer is that any possibility of an injury to the household pet must be ruled out (or a fight between two pets, if pets are present). Pets normally spread human bloodstains all around the crime scene, but the pet can be a victim, perpetrator, or witness (by the transfer of animal DNA to the perpetrator). Veterinary forensics may be needed if pets are involved. Anyway, the standard test for telling if something is human or not is called the *precipitin test*, and is a technique that is based on injecting an animal (usually a rabbit) with human blood. The rabbit's body creates anti-human antibodies, which are then extracted from the rabbit's serum. If this antiserum is then placed on a sample from the crime scene, and creates clotting, you know the sample is human. The same procedure of creating and extracting antiserum can be extended to every known animal, but most labs buy the stuff commercially rather than keep a zoo on hand.

To answer Question 4, forensic scientists must first determine if they have an adequate and quality sample. If so, direct typing (as explained previously) using the A-B-O system is done. Indirect typing would have to be done on severely dried stains, and the most common technique is the *absorption-elution test*. It is done by adding compatible antiserum antibodies to a sample, then heating the sample to break the antibody-antigen bonds, then adding known red cells from standard blood groups to see what coagulates.

To answer Question 5, forensic scientists use various color and nitrate tests, as well as heredity principles to estimate things like age, sex, and race. No exact determinations are possible, but clotting and crystallization help estimate age, testosterone and chromosome testing help determine sex, and certain (controversial) racial genetic markers involving protein and enzyme tests helps determine race.

In addition, about 80% of the population are "secretors" which means that their other body fluids contain the same antigens, antibodies, and polymorphic enzymes as in their blood. In fact, the saliva and semen in such individuals have higher concentrations of A and B antigens than their blood. The forensic serologist often will want to analyze the stains of other body fluids.

THE CRIME SCENE AND BLOOD

Wet blood has more value than dried blood because more tests can be run. For example, alcohol and drug content can be determined from wet blood only. Blood begins to dry after 3-5 minutes of exposure to air. As it dries, it changes color towards brown and black. *Blood at the crime scene can be in the form of pools, drops, smears, or crusts*. Pools of blood obviously have more evidentiary value in obtaining a wet sample. Drops of blood tell the height and angle from which the blood fell. The forensic science of blood spatter analysis says that blood which fell perpendicular to the floor from a distance of 0-2 feet would make a circular drop with slightly frayed edges. Drops from a higher distance would have more pronounced tendrils fraying off the edges (a sunburst pattern). A blood smear on the wall or floor tells the direction of force of the blow. The direction of force is always in the direction towards the tail, or smaller end, of the smear, or splatter. In other words, the largest area of the smear is the point of origin (a wave cast-off pattern). Blood crusts need to be tested with crystalline methods to make sure it's blood.

Refrigerated red blood cells have a shelf life of about 42 days, and the serum containing white blood cells can be refrigerated much longer, almost up to a year. DNA can be extracted from blood (if white blood cells which always contain a nucleus are present), and also from sperm, bone marrow, tooth pulp, and hair roots. Blood, however, is commonly used in DNA testing, as per the following steps:

1. Blood samples are collected from the victim, defendant, and crime scene
2. White blood cells are separated from red blood cells
3. DNA is extracted from the nuclei of white blood cells
4. A restrictive enzyme is used to cut fragments of the DNA strand
5. DNA fragments are put into a bed of gel with electrodes at either end
6. Electric current sorts DNA fragments by length
7. An absorbent blotter soaks up the imprint; it is radioactively treated, and an X-ray photograph (called an autoradiograph) is produced

INTERNET RESOURCES
A Bachelor's Degree Program in Forensic Serology
A Master's Degree Program in Forensic Science
Antibody Profiling (Blood Stains lead to Bar Codes)
Blood Stain Analysis (pdf file)
Catalog of Presumptive Blood Test Kits & Serology Kits
Collection and Preservation of Blood Evidence from Crime Scenes
Determining Gene Frequencies from Blood Typing [previous article]
History of Forensic Serology
Journal of American Society of Hematology
Sample Forensic Serology Report
World BloodBank Breakdown of Racial & Ethnic Distributions

PRINTED RESOURCES

Borrman, Dodd and Lincoln. (1977). ***Blood Group Serology*****, Churchill Livingstone.**
Culliford, B. (1971). ***The Examination and Typing of Bloodstains in the Crime Laboratory*****, National Institute of Law Enforcement and Criminal Justice.**
Eckert & James. (1989). ***Interpretation of Bloodstain Evidence at Crime Scenes*****, NY: Elsevier Press.**
Gaensslen, R. (1983). ***Sourcebook in Forensic Serology, Immunology, and Biochemistry*****, NIJ: NCJRS.**
Issitt, P. & C. (1977). ***Applied Blood Group Serology*****, Becton, Dickenson & Co.**
James, S., Kish, P. & Sutton, P. (Eds.) (2005). ***Principles of Bloodstain Pattern Analysis*****. Boca Raton, FL: CRC Press.**
Laber & Epstein. (1983). ***Bloodstain Pattern Analysis*****, Minneapolis: Callin Publishing.**
Moenssens, Inbau, & Starrs. (1986). ***Scientific Evidence in Criminal Cases*****, 3rd ed. Mineola, NY: Foundation Press.**
Saferstein, R. (1998). ***Criminalistics-An Introduction to Forensic Science*****, 6th ed., NJ:Prentice-Hall.**

Last updated: 02/08/06
Syllabus for JUS 425
MegaLinks in Criminal Justice