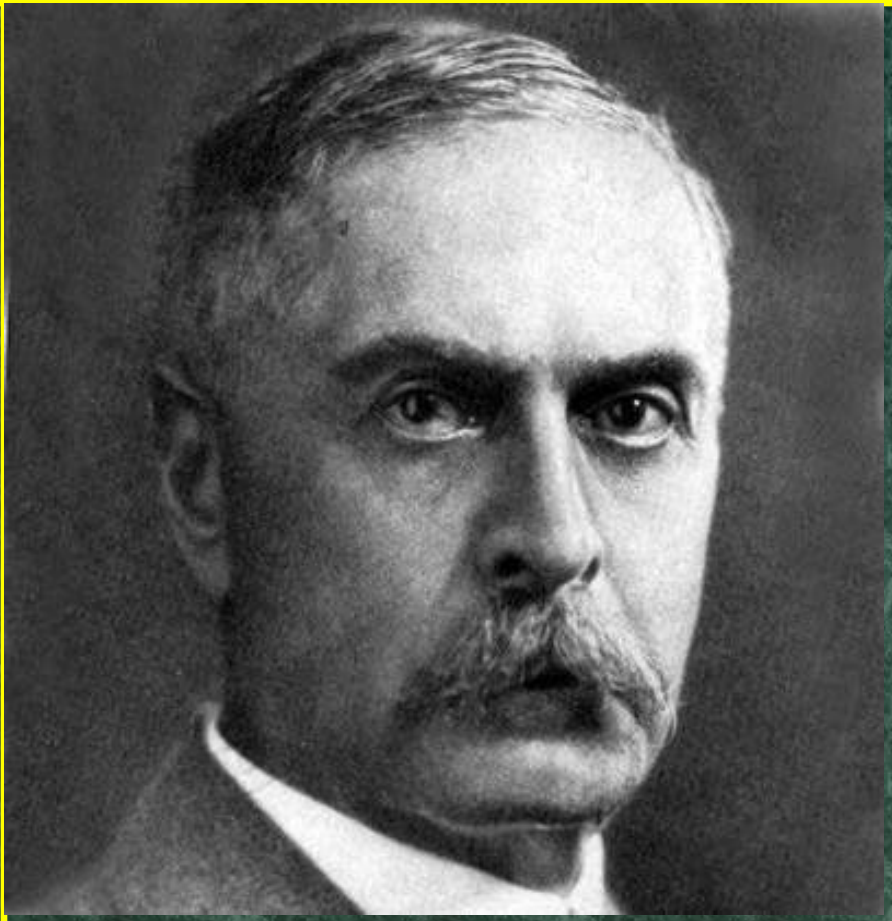
Forensic Serology

# Chapter 8 *Forensic Serology*

Courtesy of C. Fanning

- In 1901, Karl Landsteiner announced one of the most significant discoveries of the 20th Century – the typing of blood – a finding that 29 years later earned him a Nobel Prize.
- For years, physicians had attempted to transfuse blood from one individual to another, but their efforts often ended in failure because the transfused blood tended to coagulate, or clot in the body of the recipient, causing instantaneous death.
- Landsteiner was the first to recognize that all human blood was not the same; instead, he found that blood is distinguishable by its group or type.

# HISTORY of Serology



- Out of Landsteiner's work came the classification system that we call the A-B-O system. Now physicians had the key for properly matching the blood of a donor to that of a recipient. One blood type cannot be mixed with a different blood type without disastrous consequences. This discovery had important implications for blood transfusion and the millions of lives it has since saved.

# Karl Landsteiner (1868-1943)

# Karl Landsteiner

- Landsteiner's findings opened a new field of research in the biological sciences. Others began to pursue the identification of additional characteristics that could further differentiate blood. By 1937, the Rh factor in blood had been demonstrated and, shortly thereafter, numerous blood factors or groups were discovered. More than 100 different blood factors have been identified. However, the ones in the A-B-O system are still the most important for properly matching a donor and recipient for a transfusion.

# Application to Forensics

- Until the early 1990's, forensic scientists focused on blood factors, such as A-B-O, as offering the best means for linking blood to an individual. What made these factors so attractive was that in theory, no two individuals, except for identical twins, could be expected to have the same combination of blood factors. In other words, blood factors are controlled genetically an d have the potential of being highly a highly distinctive feature for personal identification.
- What makes this observation so relevant is the great frequency of bloodstains at crime scenes, especially crimes of the most serious nature: homicides, assaults, and rapes.

# Application to Forensics

- The advent of DNA technology has dramatically altered the approach of forensic scientists toward individualization of bloodstains and other biological evidence. The search for genetically controlled blood factors in bloodstains has been abandoned in favor of characterizing biological evidence by select regions of our deoxyribonucleic acid (DNA), which carries the body's genetic information. As a result, the individualization of dried blood and other biological evidence become a reality and has significantly altered the role that crime laboratories play in criminal investigation.

# Nature of Blood

- The word blood refers to a highly complex mixture of cells, enzymes, proteins, and inorganic substances.

- Plasma, which is the fluid portion of blood, is composed principally of water. The plasma accounts for 55% of blood content.

- Red blood cells (erythrocytes), white blood cells (leukocytes), and platelets (thrombocytes) are the solid materials suspended in plasma.

- Blood clots when fibrin traps and enmeshes the red blood cells. If the clotted material where removed, a pale yellowish liquid known as serum would be left.

- Antigens, usually proteins, are located on the surface of red blood cells and are responsible for blood-type characteristics.

# Antigens and Antibodies

• Red blood cells transport oxygen from the lungs to the gody tissues and remove carbon dioxide from tissues by transporting it back to the lungs, where it is exhales. For reasons unrelated to the red blood cell's transporting mission, on the surface of each cell are millions of characteristic chemical structures called antigens. More than 15 blood antigen systems have been identified, but the A-B-O and Rh systems are the most important.

•An individual that is type A has A antigens on his/her red blood cells, type B has B antigens, AB has both A and B antigens, and type O has neither A nor B antigens.

Cont.

# Antigens and Antibodies

- Another important blood antigen has been designated as the ***Rh factor***, or D antigen. People with the D antigen are said to be **Rh positive**; those without this antigen are **Rh negative**.
- In routine blood banking, the presence or absence of the three antigens - A, B, and D - must be determined in testing compatibility of the donor and recipient.

# Blood Typing

•Serum is important because it contains proteins known as antibodies. The fundamental principal of blood typing is that for every antigen, there exists a specific antibody. Each antibody symbol contains the prefix *anti-,* followed by the name of the antigen for which is it specific.

•The serum-containing antibody is referred to as the antiserum, meaning a serum that reacts against something (antigens).

•Antibodies are normally *bivalent* – that is, they have two reactive sites. This means that each antibody can simultaneously be attached to antigens located on two different red blood cells. This creates a vast network of cross-linked cells usually seen as clumping or agglutination.

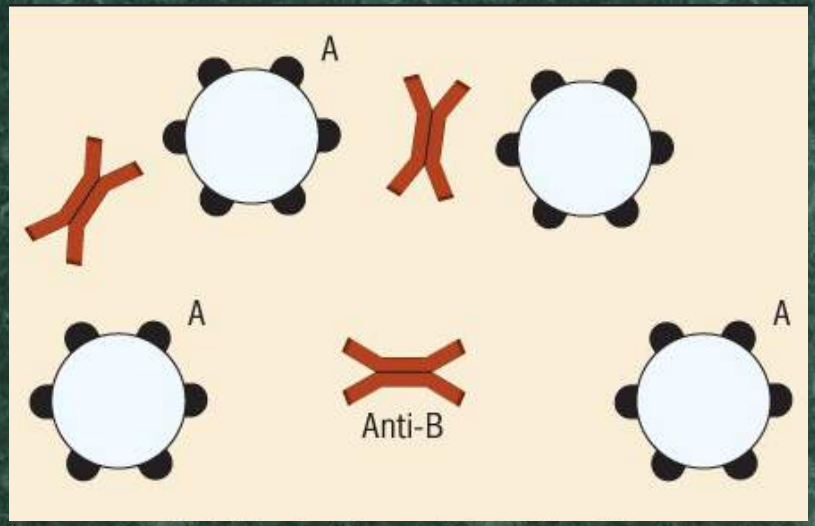
## Serology

- The term serology is used to describe a broad scope of laboratory tests that use specific antigen and serum antibody reactions.

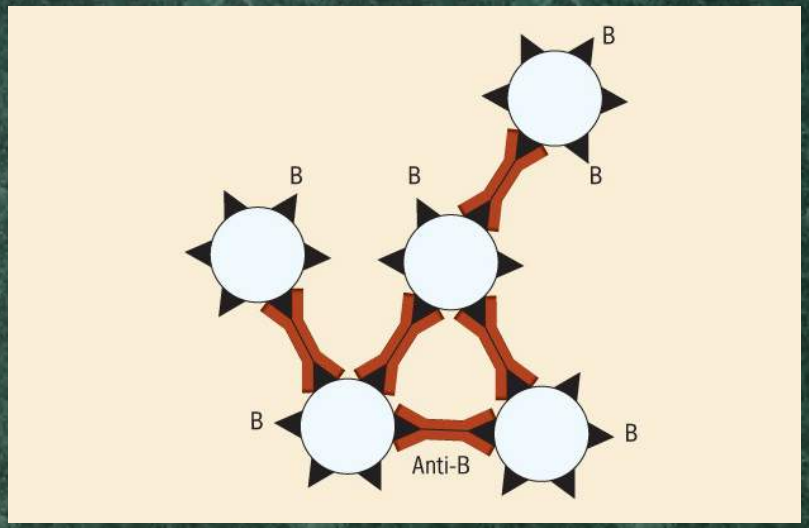
- The identity of each of the four A-B-O blood groups can be established by testing the blood with anti-A and anti-B sera.

# Serology Antigen-Antibody Reaction

- The concept of specific antigen–antibody reactions has been applied to immunoassay techniques for the detection of drugs of abuse in blood and urine.




Red blood cells containing A antigens do not combine with B antibodies.




Red blood cells containing B antigens are agglutinated or clumped together in the presence of B antibodies.

# Serology Antigen-Antibody Reaction

- Let's look a little more closely at this phenomenon. In normal blood, antigens on red blood cells and antibodies coexist without destroying each other because the antibodies present are not specific toward any of the antigens. However, suppose a foreign serum added to the blood introduces a new antibody. This results in a specific antigen-antibody reaction that immediately causes the red blood cells to link together, or agglutinate.

# Serology Antigen-Antibody Reaction

- Nature has taken this situation into account, because when we examine the serum of type A blood, we find anti-B and no anti-A. Similarly, type B blood contains only anti-A, type O blood contains both anti-A and anti-B, and type AB blood contains neither anti-A or anti-B. The antigen and antibody components of normal blood are summarized in the table given to you in your lab….
- Lab #1: Training Lab: Blood Types

- Serology involves a broad scope of laboratory tests that use specific antigen and serum antibody reactions.
- An antibody reacts or agglutinates only with its specific antigen. The concept of specific antigen-antibody reactions has been applied to techniques for detecting abused drugs in blood and urine.
- Every red blood cell contains either an A antigen, a B antigen, or no antigens. The type of antigen on one's red blood cells determines one's ABO blood type. People with type A blood have A antigens on their red blood cells, those with type B blood have B antigens, and those with type O blood have no antigens on their red blood cells.

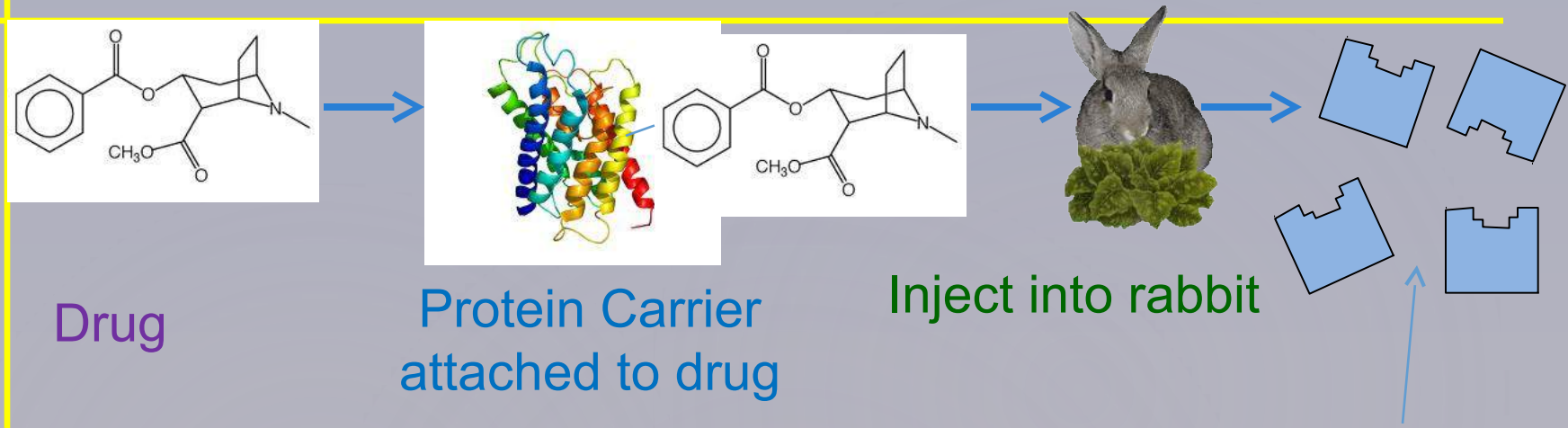
# Quick Review

# Immunoassay Techniques

- Radioimmunoassay and EMIT

# Immunoassay Techniques

- The concept of a specific antigen-antibody reaction is finding application in other areas unrelated to blood typing. Most significantly, this approach has been extended to the detection of drugs in blood and urine.
- Antibodies that react with drugs do not exist naturally; however, they can be produced in animals such as rabbits by first combining the drug with a protein and injecting this combination into the animal. This drug-protein complex acts as an antigen stimulating the animal to produce antibodies.




The recovered blood serum of the animal now contains antibodies that are specific or nearly specific to the drug originally attached to the protein carrier.

Currently, thousands of individuals regularly submit to urinalysis for the presence of abused drugs. These individuals include military personnel, transportation industry employees, police and corrections personnel, and subjects requiring pre-employment drug screening. Immunoassay testing for drugs has proven quite suitable for handling the large volume of specimens that must be rapidly analyzed for drug content on a daily basis.

## Immunoassay

- A number of immunological assay techniques are commercially available for detecting drugs through antigen-antibody reaction.
- One such technique, the enzyme-multiplied immunoassay technique (EMIT), is used by toxicologists because of its speed and high sensitivity for detecting drugs in urine.
- In a typical EMIT analysis, antibodies that will bind to a specific drug are added to the subject's urine.
- Other immunoassay procedures are also available, such as radioimmunoassay (RIA), which uses drugs labeled with radioactive tags.

# EMIT

- EMIT stands for enzyme-multiplied immunoassay technique. EMIT has gained widespread popularity among toxicologists because of its speed and high sensitivity for detecting drugs in urine.

## Antigen-Antibody Reaction

- When an animal, such as a rabbit or mouse, is injected with an antigen its body will produce a series of different antibodies, all of which are designed to attack some particular site on the antigen of interest.

## Antigen-Antibody Reaction

- This collection of antibodies is known as polyclonal antibodies.
- Alternately, a more uniform and specific collection of antibodies designed to combine with a single antigen site can be manufactured.
- Such antibodies are known as *monoclonals*.

# Forensic Characterization of Bloodstains

- Color Tests

## Forensics of Blood

- The criminalist must be prepared to answer the following questions when examining dried blood:

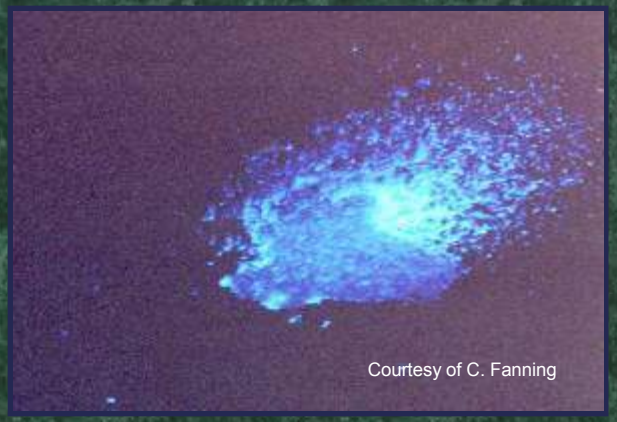
1. *Is it blood?*
2. *From what species did the blood originate?*
3. *If the blood is of human origin, how closely can it be associated to a particular individual?*

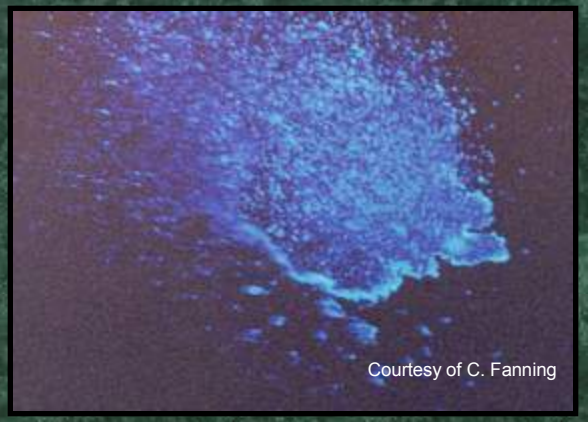
- The determination of blood is best made by means of a preliminary color test.

# Testing for Blood

- A positive result from the Kastle-Meyer (phenolphthalein) color test is highly indicative of blood. Hemoglobin causes a


- Alternatively, the luminol test is used to search out trace amounts of blood located at crime scenes.
  - Luminol produces light (luminescence) in a darkened area.




Luminol with false positive (bleach)

# Benzidine and Kastle-Meyer

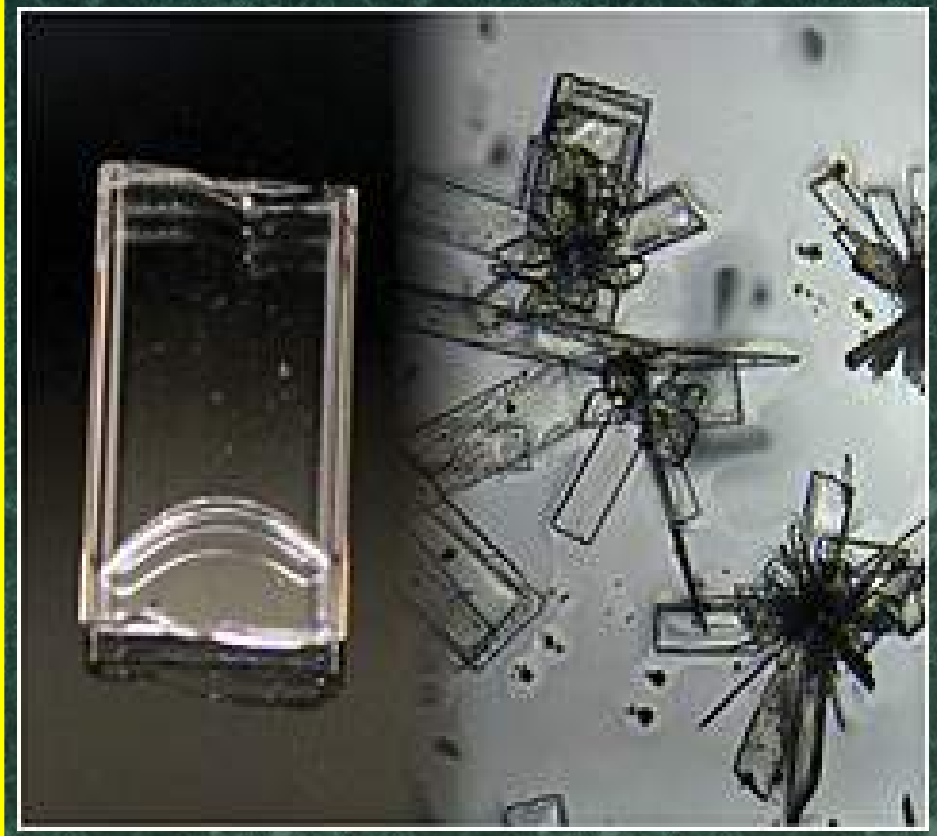
- For many years, the most common test was the benzidine color test, but because benzidine has been identified as a known carcinogen, its use has generally been discontinued and replaced with the Kastle-Meyer test.
- Both the benzidine and the Kastle-Meyer color tests are based on the observation that blood hemoglobin possesses peroxidase-like activity. Peroxidases are enzymes that accelerate the oxidation of several classes of organic compounds when combined with peroxides. For example, when a bloodstain, phenolphthalein reagent, and hydrogen peroxide are mixed together, oxidation of the hemoglobin in the blood produces a deep pink color.


# Hemastix

Field investigators have found Hemastix strips a useful presumptive field test for blood. Designed as a urine dipstick test for blood, the strip can be moistened with distilled water and placed in contact with a suspect bloodstain. The appearance of a green color indicates blood.

# Luminol and Bluestar

Another important presumptive identification test for blood is the luminol test. Unlike the benzidine and Kastle-Meyer tests, the reaction of luminol with blood produces light rather than color. By spraying luminol reagent onto a suspect item, investigators can quickly screen large areas for bloodstains. The sprayed objects must be located in a darkened area while being viewed for the emission of light (luminescence); any bloodstains produce a faint blue glow.



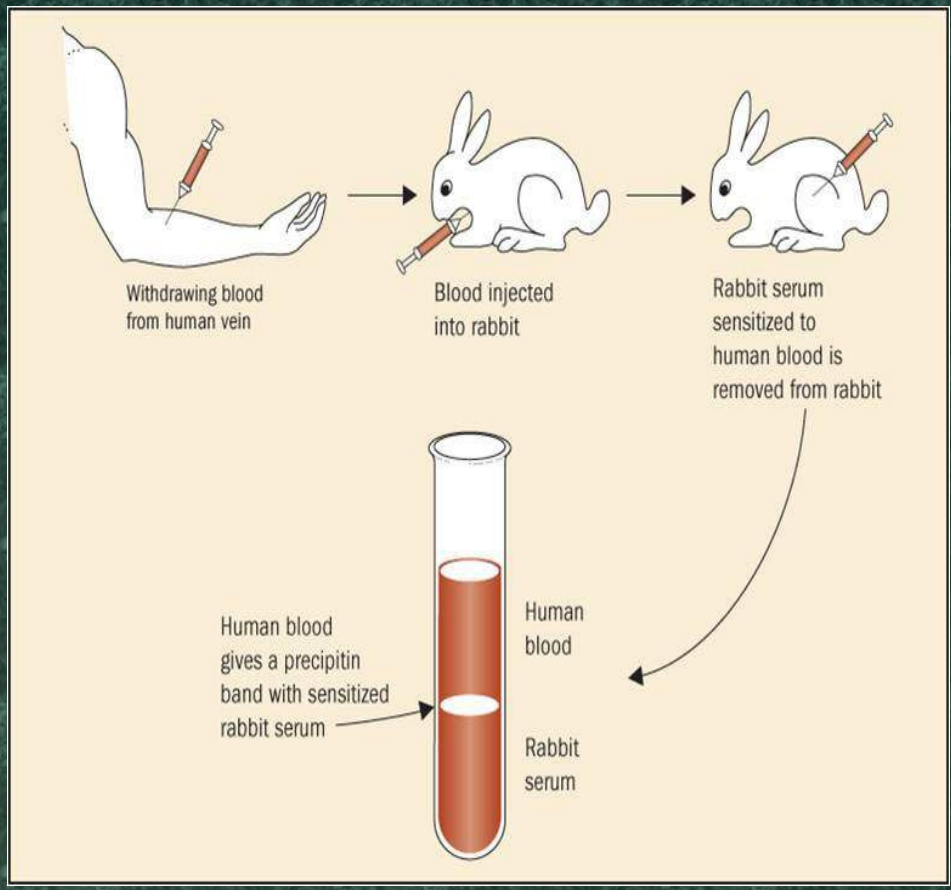
# Microcrystalline Test

The identification of blood can be made more specific if microcrystalline tests are performed on the material. Several tests are available; the two most popular ones are the Takayama and Teichmann tests. Both depend on the addition of specific chemicals to the blood to form characteristic crystals containing hemoglobin derivatives. Crystal tests are far less sensitive than color tests for blood identification and are more susceptible to interference from contaminants that may be present in the stain.

# Precipitin Test

- Once the stain has been characterized as blood, the serologist determines whether the blood is of human or animal origin. The standard test is the precipitin test. Precipitin tests are based on the fact that when animals (usually rabbits) are injected with human blood, antibodies form that react with the invading human blood to neutralize its presence. The investigator can recover these antibodies by bleeding the animal and isolating the blood serum, which contains antibodies that specifically react with human antigens. For this reason, the serum is known as human antiserum. In the same manner, by injecting rabbits with the blood of other known animals, virtually any kind of animal antiserum can be produced. Antiserums are commercially available for humans for human s and for a variety of commonly encountered animals – for example, dogs, cats, chickens and deer.

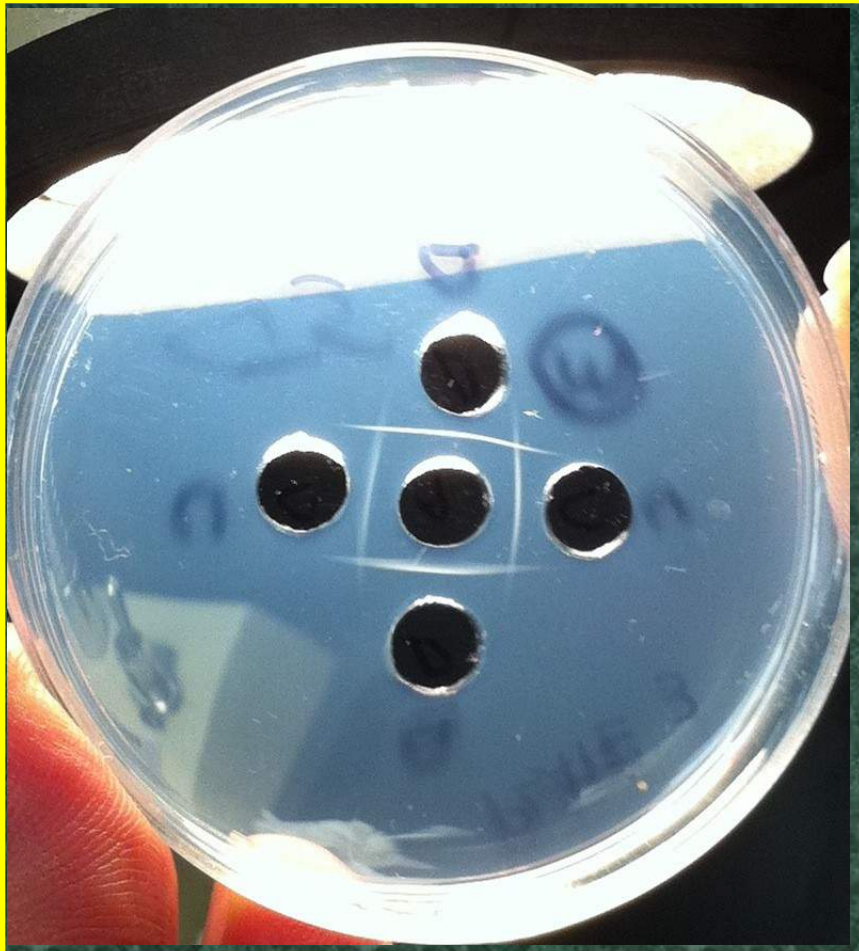
- Several techniques have been devised for performing precipitin tests on bloodstains. The classic method is to layer an extract of the bloodstain on top of the human antiserum in a capillary tube. Human blood, or for that matter, any protein of human origin in the extract, reacts specifically with antibodies present in the antiserum, and indicated by the formation of a cloudy ring or band at the interface of the two liquids.




# Precipitin Test

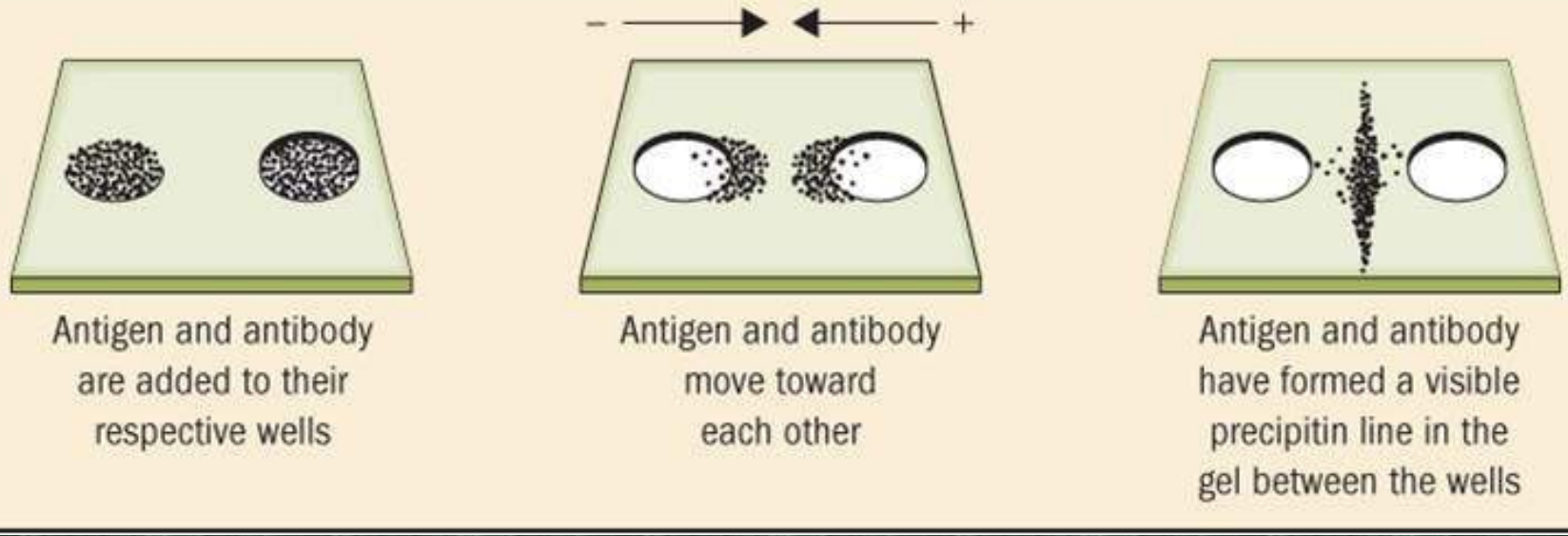
# Gel Diffusion

- Another method, called *gel diffusion,* takes advantage of the fact that antibodies and antigens diffuse or move toward one another on a plate coated with a gel medium from a natural polymer called agar. The extracted bloodstain and the human antiserum are placed in separate holes opposite each other on the gel. If the blood is human, a line of precipitation forms where the antigens and antibodies meet.



# Gel Diffusion

- Similarly, the antigens and antibodies can be induced to move toward one another under the influence of an electrical field. In the electrophoretic method, an electrical potential is applied to the gel medium; a specific antigen-antibody reaction is denoted by a line of precitation formed between the hole containing the blood extract and the hole containing the human antiserum.




The precipitin test is very sensitive and requires only a small amount of blood for testing. Human bloodstains dried for 10 – 15 years and longer may still give a positive precipitin reaction. Even extracts of tissue from mummies four to five thousand years old have given positive reactions with this test. Furthermore, human bloodstains diluted by washing in water and left with only a faint color may still yield a positive precipitin reaction.

# Precipitin Test

- Once it has been determined that the bloodstain is human, an effort must be made to associate or disassociate the stain with a particular individual. Until the mid-1990's, routine characterization of bloodstains included the determination of A-B-O types; however, the widespread use of DNA profiling or typing has relegated this subject to one of historical interest only.

# Recap

- The precipitin test uses antisera normally derived from rabbits that have been injected with the blood of a known animal to determine the species origin of a questioned bloodstain.
- Once it has been determined that the bloodstain is of human origin, an effort must be made to associate or dissociate the stain with a particular individual.
- DNA analysis has allowed forensic scientists to associate blood to a single individual.

# A-B-O vs. DNA

- Prior to the advent of DNA typing, bloodstains were linked to a source by A-B-O typing and the characterization of polymorphic blood enzymes and proteins.
- This approach has now been supplanted by the newer DNA technology.
- DNA analysis has allowed forensic scientists to associate blood and semen stains to a single individual.

# Heredity and Paternity

- The transmission of hereditary material is accomplished by means of microscopic units called genes, located on chromosomes.
- Alternative forms of genes that influence a given characteristic (such as eye color or blood type) are known as alleles.
- Paternity testing has historically involved the A-B-O blood typing system, along with blood factors other than A-B-O.
- Currently, paternity testing has implemented DNA test procedures that can raise the odds of establishing paternity beyond 99 percent.

# Testing for Seminal Stains

- Many of the cases sent to a forensic laboratory involve sexual offenses, making it necessary to examine exhibits for the presence of seminal stains.
- The best way to locate and at the same time characterize a seminal stain is to perform the acid phosphatase (an enzyme secreted into seminal fluid) color test.
  - A purple color indicates acid phosphatase enzyme.

# Testing for Seminal Stains

Semen can be unequivocally identified by either the presence of spermatozoa or of p30, a protein unique to seminal plasma.


Forensic scientists can successfully link seminal material to an individual by DNA typing.

# Rape Evidence

- The rape victim must undergo a medical examination as soon as possible after the assault.
- At that time the appropriate items of physical evidence including clothing, hairs, and vaginal and rectal swabs can be collected for subsequent laboratory examination.
- All outer and undergarments should be carefully removed and packaged separately in paper (not plastic) bags.
- Bedding, or the object upon which the assault took place, may also be carefully collected.

# Rape Evidence

- If a suspect is apprehended within 24 hours of the assault, it may be possible to detect the victim's DNA on the male's underwear or on a penile swab of the suspect.
- Items routinely collected from the suspect include all clothing, pubic hair, head hair, penile swab, and a blood sample or buccal swab for DNA typing.
- The forceful physical contact between victim and assailant may result in a transfer of such physical evidence of blood, semen, saliva, hairs, and fibers.