Body Fluids

Forensic Serology

Introduction
- Prior to the development of DNA typing, forensic serology was the primary technique of crime labs.
- Most labs still use basic serological testing procedures.
- Some do not have a DNA typing facility owing to either a lack of resources or a lack of enough cases to warrant the investment.

What is Serology?
- A term which describes laboratory tests which employ a specific antigen and serum antibody reactions
- **Serum**: the liquid that separates from the blood when a clot is formed
- **Antigen**: a substance, usually a protein that stimulates the body to produce antibodies against it
- **Antibody**: a protein that destroys or inactivates a specific antigen. Antibodies are found in the blood serum
- **Antiserum**: blood serum in which there are specific antibodies

Blood
- A complex mixture of cells, enzymes, proteins & inorganic substances
- Fluid portion of blood is called the plasma (55% of blood content)
  - primarily water (90%)
  - red cells (erythrocytes)
  - white cells (leukocytes)
  - Platelets (thrombocytes)
- **Plasma**: the fluid portion of unclotted blood
Erythrocytes
- Transport oxygen from the lungs to the body tissues
- Transport carbon dioxide from the tissues to the lungs
- Red cells possess chemical structures on their surfaces called antigens or agglutinogens
  - Agglutination: the clumping together of red blood cells by the action of an antibody

Tests for the Presence of Blood
(1 of 6)
- Police want to answer three questions:
  - Is this blood?
  - Is it from a human?
  - How closely does it match the blood of the victim or the suspect?

Tests for the Presence of Blood
(2 of 6)
- Presumptive tests for blood
  - A positive result from the Kastle-Meyer color test is highly indicative of blood.
    - Hemoglobin causes a deep pink color.
  - Alternatively, the luminol test is used to search out trace amounts of blood located at crime scenes.
    - Produces light (luminescence) in a darkened area.
  - Microcrystalline tests, such as the Takayama and Teichmann tests, depend on the addition of specific chemicals to the blood so that characteristic crystals will be formed.

Tests for the Presence of Blood
(3 of 6)
- Once the stain has been characterized as blood, the precipitin test will determine whether the stain is of human or animal origin.
- Serological tests for blood: precipitin serological test
  - Determines if blood is of human origin
  - Can be used with antiserum prepared for other animals if it is negative for human blood
  - Requires only a small blood sample
  - May produce a positive result even if bloodstains were washed down to a tiny sample remaining
  - Is highly sensitive even when bloodstains are old
- 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.

Tests for the Presence of Blood
(4 of 6)
- Serological blood typing
  - ABO system: separates human blood into four broad classifications based on the presence or absence of the antigen A or antigen B on the surface of red blood cells
  - Rh factor: expressed as positive or negative
    - Rh factor is determined by the presence of another antigen, the D antigen.
    - People having the D antigen are Rh positive; those not having the antigen are Rh negative.

Tests for the Presence of Blood
(5 of 6)
- Because blood types are inherited from a person’s parents, blood types may become concentrated among certain ethnic groups.
- 80% of the population are secretors—they have significant concentrations of antigens in other body fluids.
## Tests for the Presence of Blood

(6 of 6)

- Other blood typing systems: based on the presence of proteins in red blood cells
  - Polymorphic proteins occur in multiple forms.
  - Different forms can be identified and their statistical occurrence in the population calculated.
  - The more independent factors that can be identified in a blood sample, the smaller the percentage of the population possessing that combination of blood traits.

## Blood Type

- Three types (alleles) of blood type gene
  - A, B, O
- Each individual inherits one blood type gene from their mother & one from their father
  - 6 possible combinations (genotypes)
    - AA, BB, OO, AB, AO, and BO
- Genotype determines blood type

## Antibodies or Agglutinins

- Proteins that are present in the serum
- Responsible for ensuring that the only blood cells that can survive in a person are cells of the correct blood type
- Antibodies produced by the A alleles remove any red blood cells containing B antigens by clumping them together
- Antibodies produced by the B alleles remove any red blood cells possessing A antigens

## Type O Blood

- Possessed by people whose genotype is OO
  - both parents passed on the O gene
  - have no antigens
  - these cells can be introduced into a person with Type A or Type B because these cells are not attacked by the antibodies these people possess
  - have both a & b antibodies
  - can only have other O type cells mixed with this blood

## Type A

- Possessed by people with genotype
  - AA
  - AO
    - A is dominant to O
- Possesses antibody b
  - will destroy any Type B red cells
  - compatible with A or O red cells
Type B
- Possessed by people with genotype
  - BB
  - BO
  - B is dominant to O
- Possesses antibody a
  - will destroy any Type A red cells
  - compatible with B or O red cells

Type AB
- Possessed by people with genotype
  - AB
  - A & B are co-dominant
- Possesses no antibody
  - can have A, B, AB, or O cells added
  - Can’t be added to any other blood type without being destroyed by an antibody

Blood Typing
- Blood typing is done by reacting whole blood with antibody A and antibody B

<table>
<thead>
<tr>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Antigen Present</th>
<th>Blood Type</th>
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</thead>
<tbody>
<tr>
<td>+ Blood</td>
<td>- Blood</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>- Blood</td>
<td>+ Blood</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>+ Blood</td>
<td>+ Blood</td>
<td>A and B</td>
<td>AB</td>
</tr>
<tr>
<td>- Blood</td>
<td>- Blood</td>
<td>Neither A or B</td>
<td>O</td>
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</tbody>
</table>

Relative Frequency of Blood Types in Human Populations

<table>
<thead>
<tr>
<th>Population</th>
<th>O</th>
<th>A</th>
<th>B</th>
<th>AB</th>
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<tbody>
<tr>
<td>US whites</td>
<td>.453</td>
<td>.413</td>
<td>.099</td>
<td>.035</td>
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<td>US A-A</td>
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<td>.265</td>
<td>.201</td>
<td>.043</td>
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<tr>
<td>Chinese</td>
<td>.439</td>
<td>.270</td>
<td>.233</td>
<td>.058</td>
</tr>
<tr>
<td>Eskimos</td>
<td>.472</td>
<td>.452</td>
<td>.059</td>
<td>.017</td>
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<tr>
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<td>.499</td>
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<td>.080</td>
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<tr>
<td>Bolivian</td>
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<td>.053</td>
<td>.016</td>
<td>.001</td>
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<tr>
<td>Indians</td>
<td></td>
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</tbody>
</table>

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.

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

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.
- 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.

Stain Patterns of Blood
- The crime scene investigator must remember that the location, distribution, and appearance of bloodstains and spatters may be useful for interpreting and reconstructing the events that produced the bleeding.
- Surface texture and the stain’s shape, size, and location must be considered when determining the direction, dropping distance, and angle of impact of a bloodstain.

Stain Patterns of Blood
- Surface texture is of paramount importance. In general, the harder and less porous the surface, the less spatter results.
- The direction of travel of blood striking an object may be discerned because the pointed end of a bloodstain always faces its direction of travel.
- The impact angle of blood on a flat surface can be determined by measuring the degree of circular distortion. At right angles the blood drop is circular, as the angle decreases, the stain becomes elongated.
- The origin of a blood spatter in a two-dimensional configuration can be established by drawing straight lines through the long axis of several individual bloodstains. The intersection or point of convergence of the lines represents the origin point.

Forensic Characterization of Saliva (1 of 2)
- Characteristics of saliva
  - Consists of more than 99% water
  - pH range = 6.8–7.0
  - Contains salivary amylase (a digestive enzyme)
  - Produced in three main pairs of salivary glands: parotid, submaxillary, sublingual
  - Cleanses mouth and provides lubrication

Forensic Characterization of Saliva (2 of 2)
- Saliva is always present at the crime scene if there are bit marks on the victim.
- It can be used to identify an individual through DNA profiling.
Forensic Characterization of Semen (1 of 2)

- Characteristics of semen
  - Consists of more than 90% water
  - pH range = 7.2–7.4
- A crime scene may include a large number of items stained by semen (e.g., garments, bed clothing, rugs, drapes, solid surfaces).

Forensic Characterization of Semen (2 of 2)

- Identification of semen
  - At the crime scene: UV light
  - 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.
- 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 Collection (1 of 4)

- Conviction often hinges on the ability to link the perpetrator to the victim and the victim’s injuries.
  - Gather evidence from both the physical surroundings and the victim
  - Collect the victim’s clothing if the victim is still at the scene
  - Ensure that the victim is examined by a physician immediately

Rape Evidence Collection (2 of 4)

- Physical evidence collected from the victim:
  - Blood sample
  - Comblings from pubic hair
  - Pubic hair reference samples
  - Vaginal swab and smear
  - Rectal swab and smear
  - Fingernail scrapings
  - Oral swab
  - All clothing
  - Urine specimen
  - Head hair
Rape Evidence Collection (3 of 4)
- Saliva residues
  - Collect saliva from the victim’s skin if the assailant
    bit, sucked, or licked an area of the victim’s body

Rape Evidence Collection (4 of 4)
- Physical evidence collected from the suspect:
  - All clothing
  - Comblings of pubic hair
  - Head hair and pubic hair standards
  - Penile swab
  - Blood sample

Principles of Paternity (1 of 4)
- Nucleus: largest structure in a human cell; controls heredity
- Ribosomes: site of protein synthesis
- Mitochondria: site of energy production

Principles of Paternity (2 of 4)
- Chromosomes: hereditary material found in the nucleus
  - Egg cell: contains an X chromosome
  - Sperm: contains either an X or a Y chromosome; determines the sex of the offspring

Principles of Paternity (3 of 4)
- Chromosomes are made of nucleic acids.
  - Deoxyribonucleic acid (DNA) is the primary hereditary material.
  - DNA consists of a series of coding regions and noncoding regions that are arranged along the chromosomes.
  - Genes are sections of the DNA molecule.
- Pairs of chromosomes are considered homologous because they are the same size and contain the same information.

Principles of Paternity (4 of 4)
- When fertilization occurs, one chromosome is inherited from the mother and one is inherited from the father.
- Sons inherit their Y chromosome from their father, so paternity can often be determined by comparison of the Y chromosomes from father and son.
- Currently, paternity testing has implemented DNA test procedures that can raise the odds of establishing paternity beyond 99 percent.