Forensic Serology and DNA Analysis

- SFS3. Obtain, evaluate, and communicate information relating to biological evidence in forensic investigations.
  c. Construct an explanation to distinguish the difference between human and animal blood.
  d. Plan and carry out an investigation to analyze the physics of bloodstain patterns.
  e. Plan and carry out an investigation involving DNA processing and analysis.
What is forensic serology?

- **Forensic serology** is the detection, classification and study of various bodily fluids such as blood, semen, fecal matter and perspiration, and their relationship to a crime scene.

- A forensic serologist may also be involved in DNA analysis and bloodstain pattern analysis.
The Nature of Blood

- **Blood** is a specialized bodily fluid that is composed of two substances—**cells** and **plasma**.

- It is responsible for delivering nutrients and oxygen to and transporting wastes and carbon dioxide from cells, tissues, and organs.
The Nature of Blood

- The average human body has about 5 liters of blood (less for females, more for males).
- Blood accounts for 8% of your weight.
- Oxygenated blood is **bright red**, while deoxygenated blood is **darker red** due to the nature of **hemoglobin**.
The Nature of Blood

- **Erythrocytes** (red blood cells)
  - Contain hemoglobin and distribute oxygen
  - Account for ~93% of all blood cells
    - ~5 billion cells/L
  - Represent ~25% of all cells in the body
  - Over 2 million made every second
  - Live for about 120 days before recycled
- **Hematocrit** is a measure of RBC volume (45% of whole blood)
The Nature of Blood

- **Leukocytes** (white blood cells)
  - Function in the immune system
  - Five types categorized by immune target and function
  - Account for \(~0.1\%\) of all blood cells
    - \(~7\) billion cells/L
  - Live for 3-4 days before recycled
  - Main component of pus
The Nature of Blood

- **Thrombocytes (platelets)**
  - Responsible for blood clotting
  - Produced by fragmentation of larger stem cells
  - Account for ~7% of all blood cells
    - ~250 billion cells/L
  - “Live” for 5-9 days before recycled
  - Form long fibers to trap RBCs and form scab
THROMBOCYTE (platelet)

ERYTHROCYTE (red blood cell)

LEUKOCYTE (white blood cell)
The Nature of Blood

- **Plasma** is the fluid portion of blood and accounts for 55% of the blood content.
- It is mostly water, and it contains the clotting factors, other proteins, hormones, and various chemicals.
- **Serum** is the liquid that separates from the blood when a clot is formed (serum = plasma – clotting factors)
The Nature of Blood:
Basic Immunology

- An antigen is any substance that causes your immune system to produce antibodies against it.

- The substance may be from the environment or formed within the body.

- The immune system will kill or neutralize any antigen that is recognized as a foreign and potentially harmful invader.
Blood typing is...

- the classification of blood based on the presence or absence of inherited antigenic substances on the surface of RBCs
- More than 30 blood antigen systems have been identified (over 600 individual antigens!), but the **ABO** and **Rh** systems are the most important.
  - ABO system was discovered by Landsteiner in 1901 (Nobel Prize in 1930)
Blood Typing

- **Type A** RBCs have **A** antigens, and the blood plasma has **anti-B** antibodies
- **Type B** RBCs have **B** antigens, and the blood plasma has **anti-A** antibodies
- **Type AB** RBCs have both **A** and **B** antigens, and the blood plasma has **no** antibodies
- **Type O** RBCs have **no** antigens, but the blood plasma has **both anti-A and anti-B** antibodies
Blood Typing

- Rh factor is determined primarily by the presence of the D antigen.
- RBCs with the D antigen are Rh +
- RBCs without the D antigen are Rh -
Blood Typing

Because you have pairs of chromosomes, your \textbf{genotype} is composed of two alleles for the ABO blood group gene.

Your \textbf{phenotype} results from your specific \textbf{genotype}.

<table>
<thead>
<tr>
<th>Phenotype (blood type)</th>
<th>Genotype</th>
<th>Antibodies in serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A A \text{ or } A O</td>
<td>Anti-B</td>
</tr>
<tr>
<td>B</td>
<td>B B \text{ or } B O</td>
<td>Anti-A</td>
</tr>
<tr>
<td>AB</td>
<td>A B</td>
<td>None</td>
</tr>
<tr>
<td>O</td>
<td>O O</td>
<td>Anti-B and Anti-A</td>
</tr>
</tbody>
</table>
How common are the blood types?

In general…
- African Americans and Hispanics are more likely to have Type O blood.
- Caucasians are more likely to have Type A
- Asians are more likely to have Type B and AB blood
Blood Typing

- When an antibody comes into contact with its target antigen, clumping, called agglutination, occurs.
Blood being tested

Type AB (contains antigens A and B); agglutinates with both sera

Type B (contains antigen B); agglutinates with anti-B serum

Type A (contains antigen A); agglutinates with anti-A serum

Type O (contains no antigens); no agglutination occurs

Serum

Anti-A

Anti-B

Agglutinated RBCs
Blood Donation and Transfusion

- Because of the immune response in blood, donation and transfusion is dependent on donor and recipient blood type.

- Recipient blood should have NO antibodies that could react to the donor antigens, otherwise the blood would agglutinate in the recipient’s vessels, leading to rejection and death.
Blood Donation and Transfusion

- So, which blood type is BEST for donation?
- Which type recipient would have the easiest transfusions?
Immunoassay Techniques

- An **immunoassay** is a biochemical test that measures the presence or concentration of a substance in solutions that frequently contain a complex mixture of substances.

- A number of immunological assay techniques are commercially available for detecting drugs through antigen-antibody reaction.
Example of Immunoassay
Bloodstains at the Crime Scene

When blood is found there are four questions to ask:

1. Is it really blood?
2. Is the blood human?
3. From whom did the blood come?
4. How did the blood get there?
Is it really blood?

Kastle-Meyer Color Test

- Stain is mixed with phenolphthalein reagent and then hydrogen peroxide.
- If it is blood, the sample will turn a **bright pink** color due to hemoglobin’s oxidative effects.
- Can be a false positive since some substances have the same oxidative effects, so a confirmatory test would be needed.
Is it really blood?

**Hemastix Strips**

- Strip is moistened with distilled water and placed on blood stain.
- If blood is present, the strip will turn **green**.
Is it really blood?

Luminol Test

- Luminol reagent is sprayed onto a surface, in as dark of a room as possible.
- If blood is present a luminescent glow will show in the area of the blood, as it reacts with the iron in the hemoglobin.
- The Luminol test is very sensitive and can detect bloodstains diluted up to 10,000 times!
- Can be false positive with feces, urine, or some bleaches.
Is the blood human?

- To determine whether a blood sample is from a human or animal source, samples are tested with anti-human serum.
  - The forensic test consists of collecting the blood sample in a test tube containing an animal-derived serum containing antibodies against human blood.
  - If an insoluble complex of precipitin (this would be visible as agglutination) occurs, the test is positive for human blood.
From whom did the blood come?

- Blood typing and/or DNA testing (discussed later) can determine this.
How did the blood get there?

Dr. Herbert MacDonell, founder of the Bloodstain Evidence Institute in Corning, New York, is acknowledged as the foremost pioneer in analyzing blood patterns and has been internationally acclaimed for over five decades for his forensic expertise.
MacDonell’s Observations for Blood:

Surface texture is of paramount importance in the interpretation of bloodstain patterns, and correlations between standards and unknowns are valid only if identical surfaces are used.

*In general, the harder and less porous the surface, the less spatter results.*
MacDonell’s Observations for Blood:

The **direction of travel** of blood striking an object may be discerned by the stain’s shape.

*The pointed end of a bloodstain always faces its direction of travel.*
MacDonell’s Observations for Blood:

It is possible to determine the impact angle of blood on a flat surface by measuring the degree of circular distortion of the stain.

A drop of blood striking a surface at right angles gives rise to a nearly circular stain; as the angle decreases, the stain becomes elongated in shape.
How Bloodstain Pattern Analysis Works

Blood Drop Elongation

Angle of Impact

Blood droplet

Elongation of droplet occurs upon impact

90°  80°  70°  60°  40°  20°  10°

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MacDonell’s Observations for Blood:

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 point from which the blood emanated.*
How Bloodstain Pattern Analysis Works

Area of convergence

Blood splatter

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Other issues…

- 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 Spatter Types

- **Passive**
  
  - Created by the force of gravity

  ![Blood spatter examples](image)

  - drops
  - drip
  - flow
  - pool
Blood Spatter Types

- **Projected**
  - Occurs when some form of energy is transferred to the blood source

- Low velocity impact
- Medium velocity impact
- High velocity impact
Blood Spatter Types

- Projected

- cast-off
- arterial spurting
Blood Spatter Types

- Projected

- back spatter
- expiratory
Blood Spatter Types

- **Transfer**

  - Produced when an object with blood comes in contact with an object or surface that does not have blood contact.

  wipe
  swipe
DNA Collection & Analysis

- Investigators gather samples from the crime scene and from suspects and then analyze it for a set of specific DNA regions or markers.

- A match of one marker is not usually unique, but if a sample matches four or five markers, there is a very good chance it is a match.
DNA Collection

- DNA is collected at crime scenes in a variety of ways:
  - Sterile swabs (for moist substance)
  - Scalpels (for scraping dried substance)
  - Collection kits (samples from suspects or living victims)
DNA Collection

DNA samples can be from:

☐ Saliva
☐ Blood
☐ Hair
☐ Skin
☐ Finger or toe nails
☐ Tooth with root material

DNA Extraction
DNA Analysis

Several DNA technologies are used in DNA analysis:
- RFLP
- PCR
- STR
- mtDNA Analysis
RFLP: Restriction Fragment Length Polymorphism

- Analyzes variable lengths of DNA fragments
- Used in the development of DNA fingerprinting (Dr. Alec Jeffreys, 1985)
- Not used as much anymore because...
  - it requires a large DNA sample
  - the processing is slow and tedious
  - samples degraded by the environment do not work well with RFLP
The basic RFLP technique:

- A sample of DNA is combined with restriction enzymes that break apart the strands at specific locations.
- The resulting fragments are sorted according to their lengths by gel electrophoresis.

Gel Electrophoresis Simulation
PCR: Polymerase Chain Reaction

- **Used to make millions of exact copies of DNA from a biological sample**
- **Allows very small samples to be analyzed, such as a sample of a few skin cells**
- **Must be very careful about contamination in this process**
The basic PCR technique:

- DNA strand is heated; two pieces separate
- Each half is used as a template to build a new complementary strand

PCR Simulation
The size of the blood sample necessary for RFLP

The size of the blood sample necessary for PCR
STR: Short Tandem Repeat

- Evaluates specific regions (loci) within nuclear DNA
- FBI uses 13 standard specific STR regions for CODIS
- The purpose of establishing a core set of STR loci is to ensure that all forensic laboratories can establish uniform DNA databases and, more importantly, share valuable forensic information.
Short Tandem Repeats (STRs)

Fluorescent dye label

AATG   AATG   AATG

7 Repeats

AATG

8 Repeats

13 CODIS Core STR Loci
with Chromosomal Positions

image courtesy of National Institute of Health

TPOX
D3S1358
D5S818
FGA
CSF1PO
D7S820
D8S1179
TH01
VWA

D13S317
D16S539
D18S51
D21S11
AMEL

D13S317
D16S539
D18S51
D21S11
AMEL
Mitochondrial DNA Analysis

- Used for samples that cannot be analyzed using RFLP or STR
- Uses DNA extracted from mitochondrion rather than nuclear DNA
- Especially useful in old cases and old samples
DNA Collection & Comparison

- What happens after the samples are collected?
  - A DNA profile is created….how??
    - Markers are found by designing small pieces of DNA (probes) that will seek out and bind to complementary DNA sequences. This creates a distinct pattern. Again, one marker is not usually unique, but with four or five regions the match is likely
  - The DNA profiles are compared with samples from suspects to find possible matches.
  - If there are no suspects, a national database called CODIS may be used to find potential suspects.
DNA Collection & Comparison

- **More on CODIS:**
  - Stands for **Combined DNA Index System**
  - National network that helps identify leads for crimes with no suspects
  - Three tiers: Local (LDIS), State (SDIS), National (NDIS)
  - Uses 13 DNA regions that vary from person to person
  - Looks for matches at more than one location on a genome for more accurate results
Sources of DNA at Crime Scenes

- Cool table at:
Sources of DNA at Crime Scenes

- Examples of sources from real cases:
  - Saliva on the stamp of a stalker’s threatening letter
  - Skin cells shed on a ligature of a strangled victim
  - Perspiration on a baseball cap discarded by a rapist was compared with the saliva swabbed from a bite mark on a different rape victim
  - A single hair (without the root) found deep in a victim’s throat
  - Maggot digestive contents
How can DNA evidence be planted?

- Sneezing or coughing over evidence
- Person touches their mouth, nose or other part of the face and then touches the area that may contain the DNA to be tested.
- Scene personnel can deposit hairs, fibers, or trace material from their clothing
- Wind can carry in contaminants
Other Uses of DNA

- Paternity Testing and Proving Family Relations
- Identification of John or Jane Does
- Study of evolution and ancestry
- Studying Inherited Disorders