What is Serology?
The detection, classification, & study of bodily fluids

PART 1 - BLOOD

1. What is blood?
   a. **Plasma**
      * Fluid portion, 55% of blood volume
      * Contains: electrolytes, nutrients, vitamins, hormones, clotting factors, & antibodies
   
   b. **Erythrocytes**
      * Aka red blood cells (RBC's)
      * Carry O₂ (by hemoglobin) & CO₂
      * Anucleated (lose nuclei before maturation)
      * Most abundant cell in blood, made in marrow
   
   c. **Leukocytes**
      * Aka white blood cells (WBC's)
      * Immune system - recognize & destroy pathogens
      * Cells contain nuclei → provide DNA
      * Many types. Neutrophils most common in fighting infection
   
   d. **Thrombocytes**
      * Aka platelets
      * Clot (coagulate) together to seal a wound & prevent blood loss
      * Over 15 clotting factors in humans

2. Forensic Analysis of Blood
   Blood is **CLASS** evidence.

**STEPS TAKEN TO ANALYZE**

1. Visual Examination of Evidence

2. **Presumptive** screening test (answers the question "is it blood?

3. **Confirmatory** test ("Really, is it blood?

4. Determine **Species** origin.

5. Determine **Identity** of the blood. (Who's blood?)
### Presumptive Screening Tests

<table>
<thead>
<tr>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>* low cost</td>
<td>* may have false-positives (common w/ rust, bleach, metals)</td>
</tr>
<tr>
<td>* sensitive (reacts easily)</td>
<td>* not specific to human blood</td>
</tr>
</tbody>
</table>

**How do these work?**

- Determine if blood is present
- Use a positive & a negative control
- Add chemical to stain & look for color change

**Common Presumptive Tests:**

**Kastle-Meyer Test**
- Uses hydrogen peroxide + blood
  - $\text{H}_2\text{O}_2$ (in blood) catalyzes $\text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + \text{O}_2$
  - $\text{O}_2$ then reacts w/ color indicator of test (phenolphthalein)
  - Turns pink in presence of blood
- Subject to false positives

**Hemastix**
- Similar to Kastle-Meyer in that it uses the peroxidase activity of hemoglobin to cause a chemical change
- Comes in test-strips w/ color comparison chart

**Luminol**
- Luminol (chemical) combined w/ oxidant, then sprayed on potential bloodied area
- Chemiluminescence = emits a fluorescent glow if positive
- Pros: Sensitive, works on older stains & attempted clean ups
- Cons: Short shelf-life, bleach causes false positive, may damage DNA (if present)

**Fluorescein**
- Similar to luminol in appearance, requires an alternate light source (UV) in addition to the spray
- Pros: No false positive w/ bleach, doesn't damage DNA.

### Confirmatory Tests

- Not as sensitive, more specific to blood
- Less subject to false positives

- 2 types → **Microcrystalline**, and **Antibody Test**
  - React w/ blood & crystallizes
  - Coagulate/clot w/ blood.
**DETERMINING SPECIES ORIGIN**

1. Characteristics to look for to determine species:

<table>
<thead>
<tr>
<th>Shape of RBCs</th>
<th>Nucleus in RBCs</th>
<th>Size of RBCs</th>
<th>Presence of Platelets</th>
<th>Foa-Kuriloff Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>round or oval</td>
<td>present or absent</td>
<td>present/absent &amp; appearance.</td>
<td>WBCs found only in guinea pigs &amp; capybara</td>
<td></td>
</tr>
</tbody>
</table>

**Commonalities:**
- **Fish, Amphibians, Reptiles, Birds:**
  - oval
  - no nucleus

- **Mammals:**
  - round & concave (except camels...)
  - no nucleus

**RBC’s**

**Clotting Cells**

- platelets (fragments, no nucleus)

**Small thrombocytes w/ nucleus**

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**Mammals:** Size, Shape, Nuclei

- deer, horse, human, whale, camel

**Bird:**

- lizard

10 micron

**Non-Mammalian:**

- bird blood
- frog blood
- fish blood
Precipitin Test (confirms blood and species of origin)

What is Precipitin?
A test used to determine species of blood. It is an antibody designed to react with a species-specific antigen found in the blood.

How does it work?
A positive reaction for a specific species:
Blood sample + Antiserum = Precipitate formed (solid “clumping”)

How is it made?

Withdraw human blood sample
Inject sample into rabbit
Rabbit blood produces antibodies for human sample
Rabbit blood is sensitized to human blood
Serum from rabbit is collected

*can be made for any species*

Similar production techniques are used to make drug-tests for blood and urine samples.

Immunoadsorption
= a presumptive test for drugs in blood & urine

Drug + Protein complex
Rabbit produces antibodies in response to drug-protein complex (b/c it acts like antigen)
Recover blood serum w/ drug-specific antibodies that can be used in precipitin-like test

EMIT – Enzyme Multiplied Immunoassay Technique

1. Add Antibodies to sample (made by immunoassay to react with a specific drug).
2. Add chemically-labeled drug to sample
3. Remaining unbound labeled drugs are proportional to concentration of drug in sample

Naturally, there aren’t antibodies that react w/ drugs, so we have to make them.

Combine drug w/protein, inject into rabbit

How?
**DETERMINING WHO THE BLOOD BELONGS TO:**

- Blood Typing discovered by Dr. Karl Landsteiner in 1901
- "ABO-Rh" blood types are determined by antigens present on cell surface.

**Antigens:**
- Substance (usually **protein**) found on the **surface** of RBCs.
- Stimulate the production of antibodies
- RBCs may have **A** or **B** (or both or neither) and Rh (or not...)
- AKA "agglutinogens"

**Antibodies:**
- Blood proteins produced in response to specific antigens.
- Found in blood plasma
- Recognize "alien" substances (e.g., wrong blood type) and react with

**Blood Types:**

**GENOTYPE:** Your genes (combination of alleles) → Type A's genotype is **AA** or **AO** (A is dominant)

**PHENOTYPE:** The expression of your genes (your blood type)

Blood Transfusions must go to acceptable blood type, otherwise **AGGLUTINATION** will occur.

<table>
<thead>
<tr>
<th>Blood Type</th>
<th>Antigen</th>
<th>Antibodies in Plasma</th>
<th>Can Donate RBC's to:</th>
<th>Can Receive RBC's from:</th>
</tr>
</thead>
</table>
| A (AA or AO) | A       | Anti-B               | Type A               | Type O
|             |         |                      | Type AB              | Type A
| B (BB or BO) | B       | Anti-A               | Type B               | Type O
|             |         |                      | Type AB              | Type B
| AB (AB, only) | AB     | none                 | Type AB              | Type O
|             |         |                      | Type AB              | Type A
| O (OO only)  | Neither | Anti- A and B        | Type O               | Type O
|             | A nor B |                      | Type AB              | Type A

*Rh* factors:
- Rh+ = D
- Rh- = none

± = ±±
How We Test Blood Types:

Antiserum (Antisera):
- Serum containing antibodies
- Antibodies bind to & destroy specific antigens
- Used to determine blood type

Running the Test:
- Combine 2-3 drops of blood wi 1-2 drops of antisera
  ➔ do separate tests wi AntiA, Anti-B, & Anti-Rh
- If agglutination is observed, it is positive for that antigen

What information can we gain from blood testing?

- Your blood type...obviously.
- Possible DNA results (only if WBCs present, in tact)
  - Cells only present in FRESH blood. Once DRIED, cells LYSE
- Information on heredity
  - ABO type is inherited separately from Rh (D antigen)
  - What are the possible outcomes of offspring from parents who are A-neg and O-pos?

A-negative, rr
O-positive, oo

<table>
<thead>
<tr>
<th>BLOOD TYPE</th>
<th>ANTI-A</th>
<th>ANTI-B</th>
<th>ANTI-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-POSITIVE</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>O-NEGATIVE</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>A-POSITIVE</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>A-NEGATIVE</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>B-POSITIVE</td>
<td>R</td>
<td>R</td>
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</tr>
<tr>
<td>B-NEGATIVE</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>AB-POSITIVE</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>AB-NEGATIVE</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>INVALID</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

PT 2 - SALIVA AND OTHER BODY FLUIDS

Saliva:
- Contains water, enzymes, & mucus
- Humans produce 1-1.5 liters per day
- Aids digestion

Tests for Saliva: Looks for the presence of Amylase (enzyme in saliva breaks down starch)

- Starch-Iodine Test
  - Sample added to a starch-iodine gel (iodine stains starch blue)
  - If sample = saliva, the Amylase present will break down starch in gel & blue color disappears

- Phenolphthalein Reagent
  - Starch is linked to a colored dye if Amylase is present, dye is released.
  - Can be done wi special paper or in a test tube

Limitations:
- Amylase is present in other body fluids (highest conc. in saliva) → can't confirm
- Not very sensitive... may not detect low levels of saliva
Secretors:

- Individuals that have antigens in most body fluids (saliva, sweat, semen, gastric juice)
- 80% of humans are "secretors"
- May exhibit higher concentrations of antibodies than blood
- This means, the blood type of a secretor can be determined from any body fluid, not just blood

Semen: Identification of semen is important in forensics in order to:

- provide evidence in sexual assault cases
- help prove a crime was committed (Locard's Law)
- ID the perp -> DNA

Made of:

- Cells
  - Sperm cells, WBC's (low amount)
  - aka "spermatozoan"
  - head contains DNA, Tail (flagella) for movement, Acrosome has enzymes
- Enzymes
  - aid in maintenance, movement, & fertilization
- Organic & inorganic materials such as amino acids, sugars, ions

Confirmatory Test for Semen:

- The most reliable confirmation of semen is the presence of sperm

Confirming Semen with no Sperm:

- Oligospermia = Abnormally low sperm-count
- Aspermia = No sperm at all in seminal fluid (vasectomy)

Presumptive test for semen:

<table>
<thead>
<tr>
<th>What is Seminal Acid Phosphatase?</th>
<th>How do we test it?</th>
<th>Limitations of this test?</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;SAP&quot; - produced in prostate</td>
<td>Swab sample w/ Chemical.</td>
<td>False positives from other body fluids</td>
</tr>
<tr>
<td>Acid Phosphatases are enzymes found in various living tissues</td>
<td>positive result = PURPLE color</td>
<td>Older stains have weaker reactions (could be considered a false - negative)</td>
</tr>
<tr>
<td>Semen has high levels of Seminal Acid Phosphatase (decreases after 40)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- no relation to sperm count</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Confirmatory Tests For Semen**

**Christmas Tree Stain**
- microscopic examination of sample
- stain helps distinguish sperm cells from epithelial cells
- tails stained green, heads red

**Prostate Specific Antigen (PSA)**
- a protein made in prostate
  - aka P.S.A. or P-30
- relies on antigen (PSA)-antibody reaction
- produces antibodies in rabbit host by injecting the antigen and developing sensitized serum
- positive result = precipitate formed

Recovery of intact sperm can last anywhere from 60 - 400 hours, depending on the location.

**Search, Seizure, & the Courtroom:**
- Collect samples (body fluids, swabs, hair, combing & cut, fingernail scrape)
- Collect clothing/evidence & bed sheets (if applicable)
- Look for signs of Drug-facilitated Sexual Assault (DFSA) (blood tests, urine tests, victim's report)
- **Rape-Shield Law:**
  - past sexual behavior is barred from current testimony
  - protects the victim

**Legal Problems:**
- Backlog & major delays in physical exam - evidence collection - communication to all parties
- defining consent, identification, & "he said she said"