Serology

FRNSC 100
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Murder & Mayhem

Out damned spot! Out, I say
Here’s the smell of the blood still,
All the perfumes of Arabia will not
Sweeten this little hand. Oh, Oh, Oh!

Shakespeare

Crimes/evidence & Forensic Serology

Violent Crimes
Murder
Rape
Assault
White collar crimes
Mail fraud
Bank robbery

Forensic serology is the detection, classification and study of various bodily fluids such as blood, semen, & saliva and their relationship to a crime scene.

Serology

Serology is the first step in stain identification. Source attribution of stains will be done by DNA testing following serological examination.

Serology

Presumptive Tests are very sensitive but not specific- (i.e. will react with other compounds)
Confirmatory Tests are very specific and may be less sensitive

- Blood Identification
  - Presumptive Test(s)
    - Phenolphthalein
    - Luminol
  - Confirmatory Test
    - Takayama
Serology

- Semen Identification
  - Presumptive Test
    - Acid phosphatase
  - Confirmatory Test
    - sperm
    - P30

- Saliva Identification
  - Presumptive testing
    - Amylase

Blood

Blood is a highly complex mixture of cells, enzymes, proteins, and inorganic substances.

- Plasma - the fluid portion of blood composed principally of water.
- Red blood cells (erythrocytes)
  - Antigens, usually proteins, are located on the surface of red blood cells and are responsible for blood-type characteristics.
- White blood cells (leukocytes)
  - Contains DNA
- Platelets are the solid materials suspended in plasma.

Blood Identification

Presumptive test chemical test(s) which uses color change to detect the possible presence of blood.

Blood- Purpose

- Supplies oxygen to tissues (bound to hemoglobin, which is carried in red cells)
- Supplies nutrients such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins (e.g., blood lipids))
- Removes waste such as carbon dioxide, urea, and lactic acid
- Responsible for immunological functions, including circulation of white blood cells, and detection of foreign material by antibodies

Blood- Purpose

- Coagulation - part of the body’s self-repair mechanism (blood clotting after an open wound in order to stop bleeding)
- Transport of hormones
- Regulation of body pH
- Regulation of core body temperature

Phenolphthalein Test (Kastle-Meyer)

The Kastle-Meyer test relies on the iron in hemoglobin, which is the iron-containing portion of a red blood cell, to promote the oxidation of phenolphthalein to phenolphthalein. Phenolphthalein is colorless, but in the presence of blood and hydrogen peroxide, it changes to phenolphthalein, which makes the solution pink.

- The names of the two chemicals—phenolphthalein and phenolphthalein—are very similar, but they are structurally different.

\[
\text{Fe}^{4+} + \text{phenolphthalein} + \text{H}_2\text{O}_2 \rightarrow \text{phenolphthalein (pink)} + \text{H}_2\text{O} + \text{Fe}^{3+}\]

- This test is very sensitive. One drop of blood diluted in 10,000 drops of water can still be detected by the Kastle-Meyer test.
Phenolphthalein Test (Kastle-Meyer)

This test is very sensitive. One drop of blood diluted in 10,000 drops of water can still be detected by the Kastle-Meyer test.

- This is a presumptive test. It will give a false positive result:
  - in the presence of vegetable peroxidases, such as those in horseradish, broccoli, cauliflower, etc.
  - other oxidizing species in the sample will also cause a false positive.
  - chemical oxidants such as copper and nickel salts will cause the Kastle-Meyer reagent to turn pink before the addition of the hydrogen peroxide, thus it is vitally important to add the reagent first, then wait a few seconds, then add the hydrogen peroxide.

Luminol

Luminol (C8H7N3O2) is a chemical that exhibits chemiluminescence, with a striking blue glow, when mixed with an appropriate oxidizing agent—like heme in blood—thus it may be used to locate traces of blood, even if it has been cleaned or removed.

- The solution of luminol and the activator are sprayed on the area under investigation. The iron present in any blood in the area catalyzes the chemical reaction that leads to the luminescence revealing the location of the blood.
- The amount of blood necessary for the reaction to occur is very small relative to the amount of luminol, allowing the detection of even trace amounts of blood.
- The glow lasts for about 30 seconds and is blue. Detecting the glow requires a fairly dark room. Any glow detected may be documented by a long exposure photograph.

- This is a presumptive test. It will give a false positive result and it can be triggered by a number of substances such as:
  - copper or copper-containing alloys,
  - certain bleaches; and, as a result, if a crime scene is thoroughly cleaned with a bleach solution residual cleaner will cause the entire crime scene to produce the typical blue glow, effectively camouflaging any organic evidence, such as blood.
  - small amounts of blood present in urine, and it can be distorted if animal blood is present in the room that is being tested.
  - fecal matter, causing the same glow as if it were blood.
  - It may prevent other tests from being performed on a piece of evidence.

Ludwik Karol Teichmann (1823-1895)

- Polish physician/chemist
- In 1853, published a paper on the crystallization of certain organic compounds of the blood, describing the preparation of the microscopic crystals of hemin (heme chloride).
- First test for the presence of blood in suspect stains on clothes, furniture, or other objects.
- (for blood) the suspected liquid is put under a coverglass with a crystal of sodium chloride and a little glacial acetic acid; heat carefully without boiling and then cool. If blood is present, rhombic crystals of hemin will appear.
Takayama Test
Confirmatory Test for Blood

Takayama is a crystal test used for the confirmation of the presence of blood on samples that screened positive a presumptive test.

• Reacts with Hemoglobin in the blood

Karl Landsteiner

Vienna physician and professor, 1901-1909 working with blood transfusions that some individuals blood “clumped”.

• Discovered that the blood can be typed into different groups. These groups are eventually labeled as types, A, B, AB, and O.

• First suggested these could be used to establish paternity

• Antibodies and antigens

• Introduce chemistry into serology

1930- awarded the Nobel Prize

ABO Typing System

A blood type (also called a blood group) is classification of blood based on the presence or absence inherited substances on the surface of red blood cells (RBCs) and the presence of antibodies in the plasma.

Leon Lattes (1887-1954)

• Lattes thought that ABO blood typing might be useful for the identification of individuals

• Professor at the Institute of Forensic Medicine at the University of Turin in Italy,

• 1915 he developed procedure for determining the blood type (ABO) of a dried blood stain,

• Applied it to criminal investigations

Type A will have A antigens on RBC and anti B antibodies in serum

Type B will have B antigens on RBC and anti A antibodies in serum

Type AB will have A & B antigens on RBC and no antibodies in serum

Type O will have no antigens on RBC and A & B antibodies in serum

People with Type O blood are considered the “universal donors” because their blood can be transfused into anyone without causing reactions.
Why was ABO typing of dried stains used in criminal investigations?

- ABO typing can be used to “sort” people into four groups.
- Reference blood samples are typed and compared to unknown blood stains.
  - Type is different - reference is excluded as a possible source of the stain.
  - Type is the same - reference is a possible source of the stain but not conclusive because other people also have the same type.
- Statistics needed to be reported with the result.

Rape & Sexual Assault Cases

In 2007, there were 248,300 victims of rape, attempted rape or sexual assault (down from 272,350 in 2006).

Only 3% of the perpetrators are strangers. >100,000 cases per year across the U.S.

Types of Sexual Assault Evidence

- **Swabs**
  - Vaginal, anal, oral, surface and penile
  - Generally have multiple sources.

- **Slides**
  - Confirm the presence of sperm

- **Pubic combings**
  - Roots v shafts ... microscopy v DNA analysis

- **Articles of clothing**
  - Bed sheets, undergarments, towels/wash cloths
  - Alternate light sources for finding stains

Semen

Semen is an organic fluid, also known as seminal fluid, that may contain spermatozoa (sperm). Finding the presence of sperm is a confirmatory test for semen.

In humans, seminal fluid contains several components besides sperm:

- **proteolytic and other enzymes such as acid phosphatase (AP).**
  - The level of AP activity is 500 to 1000 times higher in human semen than in any other normal body fluids or secretions. The presence AP is sometimes used for presumptive testing for semen

- **Prostate Specific Antigen (PSA)** (also known as p30) is present in small quantities in the serum of men with healthy prostates, but is often elevated in the presence of prostate cancer and in other prostate disorders. The presence of PSA (p30) is used for confirmatory testing for semen
• **Head**
  - Contains the nucDNA

• **Mid-Section/Neck**
  - Contains mtDNA which supplies energy to the “cell”

• **Tail**
  - Protein fibers that contract and release on alternate sides to create the swimming motion
  - ~1000 complete movements to swim 1.25 cm

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**Influences on Finding Sperm**

- Vasectomy or low sperm count
- Minimal ejaculation
- Urination
- Vaginal pH
- Contraceptive usage (e.g., condom)
- Bathing

**Survival of Sperm**

- It’s important that samples are collected as soon as possible, as sperm survive for different periods of time depending on the environment they are found in ...
  - ~72 hours to 7 days in the vaginal cavity
  - ~24 hours to 3 days in the anal cavity
  - ~8-24 hours in the oral cavity

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**Staining Sperm Cell Slides**

- **H & E Staining (Hematoxylin and Eosin)**
  - Hematoxylin, a basic dye, binds to acidic components of a tissue (such as DNA and RNA) ... usually a **blue or purple color**
  - Eosin, an acidic dye, binds to basic components of a tissue ... usually a red or pink color

**Prep for Microscopy**

- To make searching for sperm easier:
  - Extraction buffer plus SDS and Pro K may be added to a sperm sample to lyse any non-sperm cells (epithelial, white blood)
  - Sperm heads are largely unaffected
  - Sperm tails can be “lysed” or stripped from the sperm “cell”
Staining Sperm Cell Slides

Semen Identification

Simple test
- Extract stain into a buffer
- Place drop on test strip in “S” well
Negative: no color
Positive: pink color

ABAcards
- Detects the presence of p30 protein in seminal fluid (named because it weighs 30,000 daltons)
  - p30 is 200,000 to 5.5 million nanogram per ml of semen.
  - The sensitivity of ABAcards® p30 test is 4 ng/ml
- p30 reacts with “mobile” monoclonal anti-human and forms “mobile” Ab+Ag complex
- Ab+Ag migrates to test area “T” and binds with immobilized antibody (anti p30)
- When Ab+Ag +Ab > 4 ng/ml pink dye particles colored band in the test area “T”
- If no p30 is present, mobile Ab migrates to “C” area, is immobilized and forms a pink band to show test is working.

Saliva
- Saliva is rich in the enzyme alpha-amylase (a.k.a. α-amylase, salivary amylase), an enzyme that breaks down complex carbohydrates into smaller sugar molecules.
- The presence of amylase is presumptive for saliva as it is also present in fecal material and vaginal secretions.
- Amylase is an enzyme and may become “inactive”. This would give a false negative reaction for the presence of saliva.
- Presumptive tests include:
  - Starch-Iodine test for amylase
  - Phadebas
  - Rapid Stain Identification (RSID) Saliva

Starch-Iodine test
Amylase will breakdown starch. Iodine is added and will detect starch that has not been hydrolyzed.
- Amylase radial diffusion assay
  - Pour agar gel containing starch into culture plate
  - Create wells in solidified agar with vacuum punch
  - Add extract of evidence sample to well; Add standards to other wells (amylose samples at known concentrations)
  - Incubate overnight at 37 degrees C
  - Stain with iodine solution
- Not specific test for amylase

Phadebas
Phadebas tablets consist of insoluble starch polymers to which a blue dye has been covalently bound
- Extract sample and add tablet
- When amylase is present:
  - Polymers are degraded
  - Dye is liberated and becomes soluble
- Concentration of soluble dye is measured by spectrophotometry at 620 nm
Rapid Stain Identification (RSID) Saliva

- One such test is the RSID-Saliva™ kit which is a lateral flow chromatographic strip test designed to detect α-amylase, an enzyme present in human saliva.
- Designed to work like the p30 test.