Forensic Serology

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Forensic serology is the application of the study of blood, semen, saliva and other body fluids, to legal matters. The field generally is comprised of the detection of enzymes and antigens, as in the identification of seminal stains or blood typing (ABO and secretor status) and DNA typing.

The serology section of a forensic laboratory may deal with any or all of the following:

- **blood typing**
- **characterization of unknown stains**
- **blood spatter analysis for crime reconstruction**
- **paternity testing**
- **semen identification in sexual assault cases**
- **DNA typing for identification of victims and suspects**

The Composition of Blood

Blood is a mixture of many components:

cells **inorganic substances (salts)**
enzymes **water**
proteins

55 % of blood content is plasma – mostly water and substances dissolved in it

Most of the solid materials (by weight) are cells

red blood cells, RBCs (erythrocytes)

white blood cells (leukocytes)

Forensic Characterization of Bloodstains

Three questions that must be answered by the forensic investigator:

1) Is it blood? Use *presumptive* tests:

Kastle-Meyer
Leucomalachite Green
Luminol

2) If yes, is it human blood? (Precipitin Test)

3) If yes, can it be associated with an individual? (DNA)

Is It Blood? Presumptive Tests for Blood

Presumptive tests rely on hemoglobin's ability to catalyze the oxidation of certain reagents, usually resulting in a color change. Oxidizing agent is usually hydrogen peroxide (H_2O_2).

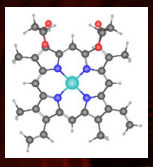
Visible Stains

Kastle Meyer: solution of phenolphthalein is applied to suspected blood stain, followed by hydrogen peroxide. A bright pink color indicates the presence of blood.
Leucho-Malachite Green: same procedure as K-M; produces blue-green color in the presence of blood.

Invisible Stains

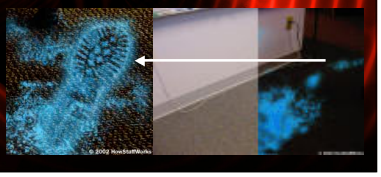
Luminol: used for large areas where blood is suspected but not visible. If blood is present, it will glow. Must be viewed in darkness and photographed quickly.

Luminol



Heme structure

- **Red blood cells contain hemoglobin (Hb) – the protein responsible for transporting oxygen**
- **Each Hb contains four iron (Fe) containing hemes**
- **Luminol reacts with iron**



False Positives

Blood is not the only material that will cause presumptive test reagents to change color.

Horse radish & potatoes can also give positive results (these are unlikely to be at a crime scene, though).

Luminol can give false positive results when exposed to household bleach, some metals, and some vegetables.

Blood is the only body fluid that will give a positive reaction.

Positive results must be confirmed with Precipitin Test.

Confirmatory Tests

Once a presumptive test indicates that a stain may be blood, the serologist must confirm that it is human blood.

Precipitin test identifies the presence of proteins that are found only in human blood.

Precipitin Test Procedure

- animal (usually a rat or rabbit) is injected with human blood
- animal's blood forms antibodies
- antibodies are harvested from animal's blood serum ("antiserum")
- in a test tube, an extract from the suspected bloodstain is added to the antiserum
- if a precipitate forms where the two meet, it is human blood

Blood Typing: Antigens

On the surface of red blood cells are molecules (proteins) called antigens.

Antigens allow a living system to recognize foreign biological substances, such as infectious agents and allergens.

Antigens also impart specific characteristics to the RBCs. These characteristics can be partly used to identify individuals.

Human RBCs have many antigens, but the ones most commonly used for blood typing are Rh & ABO.

Blood Typing

Blood typing involves determination of the antigens present on an individual's RBCs.

type A blood – contain "A" antigen on RBCs

type B blood – contain "B" antigen on RBCs

type AB blood – contains both A and B antigens

type O blood – contain no A or B antigens

Rh+ blood – contain Rh antigen

Rh- blood – no Rh antigen

Distribution of Blood Types in the US

A blood type (40-42%) is most common among Caucasians and those of European descent.

B blood type (10-12%) is most common among African Americans and Thai Asians.

AB blood type (3-5%) is most common among Japanese & Chinese populations.

O blood type (43-45%) is most common among indigenous peoples and Latin Americans.

Blood Typing

Blood also contains antibodies – proteins that recognize and bind to certain, specific antigens.

Blood Type	*Antigens on RBC*	*Antibodies*
A	A	anti-B
B	B	anti-A
AB	AB	none
O	none	both A & B

A agglutinates with anti-A; B agglutinates with anti-B; AB agglutinates with both anti-A & anti-B; O will not agglutinate with either serum.

To determine blood type, observe: Does the sample agglutinate in response to the antiserum added?

Blood Typing

Human serum containing specific antibodies can be purchased.

Separately mix a drop of unknown blood sample with drop of each anti-serum.

Reaction between blood and anti-serum

No reaction between blood and anti-serum

Blood Typing Example

A sample of unknown blood is mixed with three anti-sera samples:

Tube 1 (Anti-A): No reaction

Tube 2 (Anti B): No reaction

Tube 3 (Anti Rh): Cloudy reaction

In terms of the A-B-O and Rh systems, what type blood is the sample? O positive

The ABO antigens are not confined to RBC; 80% of Americans are "secretors", which means that they secrete their ABO antigens into other body fluids.

Characteristics of Blood

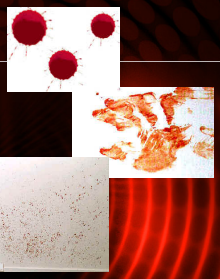
CLASS
- Species
- Type
- Disease
- Rh factor
- Antigens

INDIVIDUAL
- DNA

Is *color* a class or individual characteristic of blood?

THINK ABOUT IT.

Bloodstain Analysis



Categories of Bloodstains:

Passive (dripping)

Transfer (smearing)

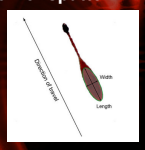
Projected

Projected bloodstains occur in shootings, trauma from blunt weapons, hacking, beating or slashing attacks.

Projected Bloodstain Analysis

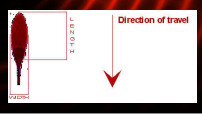
Two Important Determinations:

a. direction of spatter



b. angle of impact with surface

sin α = (width drop / length drop)



Forensic Characterization of Semen

Many crimes involve sexual assault. Forensic Investigators may need to search for semen stains at a crime scene.

Bedding, clothing, towels, carpets, cushions, vehicle seats, etc.

Seminal Fluid contains:
- water, spermatozoa, enzymes, inorganic salts

Presumptive Tests
- UV light (semen fluoresces under UV light)
- Acid Phosphatase (a presumptive test reagent that rapidly turns purple if AP is present)

False Positives

Acid phosphatase (AP) is present in many substances, but it is in especially high concentrations in seminal fluid.

Some fruit juices, contraceptive creams, and vaginal secretions will also produce a purple color with AP test.

However, the color change is *much slower* with these substances.

Semen will produce the color change very rapidly (fresh stains will take ~10 seconds, older stains may take ~30 sec -- and always less than 1 min).

A positive result for AP determines not the presence of AP, but that it is present at a certain concentration

Confirmatory Test Required

1) Microscopic visualization of sperm
   A normal ejaculate is 2-5ml and contains 100-150 million spermatazoa
2) Prostate Specific Antigen (PSA) (a protein found exclusively in seminal fluid

Forensic Characterization of Saliva

Saliva is a mixture of many components:

- 99% water
- Mucin (protein helps in swallowing)
- Amylase (enzyme to help digest carbohydrates)
- Buccal cells (good source for DNA)

Adults produce 1.0-1.5 liters of saliva/day. Saliva is not uncommon at crime scenes, especially those involving sexual assault and bite marks.

A simple test for saliva involves mixing starch, iodine, and a sample of the presumed saliva together. Starch and iodine are a deep blue color when mixed together. The amylase breaks down starch, however, and the color fades (takes about 15 mins @ 37 °C).

Conclusions

Unknown Stain

KMG, LG, Luminol	AP, P-30	Amylase
Blood	Semen	Saliva
↓	↓	↓
Physical Pattern	Microscopy	DNA
↓	↓	
DNA	DNA	