**Blood as Evidence**

Blood is a valued form of evidence in violent crimes such as murder and rape. Its characteristics are variable among the population making it possible to rule in or rule out a person as the perpetrator. Stains and blood spatter patterns can also reveal information about the crime. Forensic serology is the branch of forensic science that involves processing of blood stains and spatter patterns. Before the 20th century, there was very little research in this area and only a couple of tests for blood had been developed: guaic test (by Van Deem) and the hydrogen peroxide test. (by Schonbein). Mathieu Orfila had suggested that blood could be analyzed using a microscope.

**What is Blood?**

The major components of blood are:

1. **Plasma/Serum**: the pale, yellow colored liquid portion that contains \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_, \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_, \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ and dissolved nutrients.

**55% of the total volume is plasma**. **45 % is formed elements** such as blood cells and platelets.

1. **Red Blood Cells**: carry \_\_\_\_\_\_\_\_\_\_\_\_\_\_to cells and \_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_away from the cells to the lungs to be exhaled.

Blood gets its red color from\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_, a protein that is needed to carry iron. The red blood cells contain hemoglobin and iron.

1. **White blood cells**: cells responsible for immunity, antibody production in the lymph nodes, and fighting infection. There are three basic types of white blood cells:
   1. Lymphocytes
   2. Monocytes
   3. Neutrophils
2. **Platelets:** tiny fragments of cells responsible for initiating and participating in\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_.

**Blood Types**

1901: Karl Landsteiner discovered that it is not possible to transfuse blood from any one human into any other human. He searched to find out why some people lived after a transfusion and some died. Why did the transfusions fail? His search for the answer led him to conclude that human blood can be classified into a few types known as the ABO system. These types are A, B AB, and O based on the presence or absence of specific proteins called antigens.

Early 1970’s: Forensic science began to utilize these blood groupings for clues to link blood to an individual. Blood type is considered \_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_because it is not unique to an individual the way that DNA or fingerprints are.

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| --- | --- | --- |
| **Blood Type** | **Antigen on RBC** | **Antibody in plasma** |
| Type A | A antigen | B antibody |
| Type B | B antigen | A antibody |
| Type AB | A and B antigens | No antibody |
| Type O | No A or B antigen | Both A and B antibodies |

The type of the blood represents the antigens present on the red blood cell. A person with Type A will have the \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_on their red blood cells. A person with \_\_\_\_\_\_\_\_\_\_\_ will have the B antigen. Type AB will have both the A and the B antigen. The A and B antigens are absent on the cells of Type of blood. Rh factor is another antigen that helps to distinguish between blood samples. Rh positive means that the antigen is present. Rh negative means that the antigen is not present.

Example: Blood that is A positive has both the A antigen and the Rh antigen present on the cell. Blood that is A negative has the A antigen present but lacks the Rh antigen.

**Frequency of Blood Types**

Blood types vary within the human population but the rates of occurrence correlate with race. Blood type can be used to link an individual to evidence but there are better methods of matching an individual to blood such as DNA.

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| **Blood type** | **Expected Frequency** |
| A positive | 1 in 3 |
| A negative | 1 in 16 |
| B positive | 1 in 12 |
| B negative | 1 in 67 |
| AB positive | 1 in 29 |
| AB negative | 1 in 167 |
| O positive | 1 in 3 |
| O negative | 1 in 15 |

**Correlation of Blood Type and Race**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Type | African American | Asian | Caucasian | Hispanic |
| O positive | 47% | 39% | 37% | 53% |
| O negative | 4% | 1% | 8% | 4% |
| A positive | 24% | 27% | 33% | 29% |
| A negative | 2% | 0.5% | 7% | 2% |
| B positive | 18% | 25% | 9% | 9% |
| AB positive | 4% | 7% | 3% | 2% |
| AB negative | 0.3% | 0.1% | 1% | 0.2% |

Determination of blood type is based on an antigen-antibody reaction. When the antigen for a given type is exposed to the antibody against that antigen, \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_or clumping of the cells occurs. This is the reason why Landsteiner’s blood transfusions resulted in death for some recipients. If a recipient with Type A blood received blood containing the antibody to Type A (A antibody) then the cells would clump and the recipient would die.

**Using Blood Type to Solve a Crime**

Blood type can be used to \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_down a pool of possible suspects. If the unknown blood type is A positive then every other blood type can be excluded. The presence or absence of \_\_\_\_\_\_\_\_\_\_\_\_adds another layer of inclusion or exclusion.

Other antigens present on the red blood cells**: Duffy, Kidd, MNS, Kell, Lutheran**

White blood cells have HLA or\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_.

**Blood Stains**

Determine if the stain is blood or some other red substance.

Blood released from the body is \_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_for about the first 3 to 5 minutes. As it dries it turns \_\_\_\_\_\_\_\_\_\_\_ to \_\_\_\_\_\_\_\_\_\_\_. Wet blood is usually easier to test than dried blood.

In the field the CSI must have a quick, easy and affordable method to determine if a stain is possibly blood. A \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_ is a quick test that can be performed at the scene to determine if a piece of evidence is relevant and needs further testing. This saves time and money.

**Presumptive Tests for Blood**

**Adler’s or Benzidine Test:** mix hydrogen peroxide with benzidine and treat the stain. An \_\_\_\_\_\_\_\_\_\_\_\_\_-\_\_\_\_\_\_\_\_\_\_\_\_\_\_ reaction takes place and the benzidine is reduced. A \_\_\_\_\_\_-\_\_\_\_\_\_\_ color indicates a positive test. This test has been banned by the EPA because benzidine is a carcinogen.

**Kastle-Meyer Test:** potassium hydroxide, phenolphthalein and zinc dust are mixed and the stain is treated. Kastle-Meyer reagent combined with hydrogen peroxide and blood causes the hemoglobin in the blood to catalyze and the indicator turns a \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_(positive test). If vegetable materials are present, a false positive result can be obtained.

**Hemastix test:** cellulose strip containing a mixture of \_\_\_\_\_\_\_\_\_\_\_\_\_ and hydrogen peroxide on a tiny pad. Moisten the strip and dip or rub the sample to be tested. Hemoglobin catalyzes the conversion of o-toluidine to a \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ colored product (positive test). The intensity of the green color is matched to a scale to indicate the concentration of blood present in the sample.

**Luminol: 5-amino-2,3-dihydro-1,4-phthalizinedione**

First developed by Walter Specht in 1937. Luminol and hydrogen peroxide in the presence of blood will react and one ring of the luminol breaks apart. Nitrogen gas is released and 3-aminophthlate is produced in an excited state. After a brief moment, the 3-APA gives off a photon (425nm). A \_\_\_\_\_\_\_\_\_\_fluorescent light is given off.

Luminol is highly sensitive and will detect blood stains diluted up to 10 million; works well on old blood stains. It does not interfere with DNA analysis or blood typing.

False positives can be obtained with plant enzymes, oxidizers, metals, and chlorine.

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| **Presumptive Test** | **Indication of Positive** | **Situation Used** | **False Positives** |
| Phenolphtalein (Kastle-Meyer) | Bright pink color | on visible stains | Vegetable material (potatoes and horseradish) |
| Tetramethylbenzidine (TMB) / Hemastix | Green to blue-green color | on visible stains | Oxidizing agents, catalyst, vegetable peroxidase, cosmetics |
| Luminol | Blue-white to yellow-green light | latent blood | Plant enzymes, oxidizing agents, metals, chlorine |
| Fluorescein | Fluoresce with UV light source | latent blood, vertical surface | Copper, hypochlorite |

**Confirmatory Tests for Blood**

**\*\*\**For any presumptive test that is positive, a confirmatory test must be done to correlate the results and rule out a false positive presumptive test. \*\*\*\*\*\****

**Takayma Test (1912) :** The reaction of pyridine and blood causing the reduction of hemoglobin to a pyridine-hemoglobin complex that is a \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ color.

Teichmann Test (1853): a solution of glacial acetic acid and sodium chloride is mixed with blood; the hemoglobin molecules split producing crystals of hemin. (purple to almost black).

**Human blood or animal blood: How can you tell the difference?**

Once a stain tests positive for blood with both the presumptive test and a confirmatory test, the origin of the blood must be determined: animal or human

**Ouchterlony Test (precipitin test; 1960):** test is based on an antigen-antibody reaction. An experimental animal (usually a rabbit) is injected with a sample of human blood. The rabbit makes antibodies to the human blood cells. Blood is drawn from the rabbit and mixed with the unknown blood sample. If the blood is human, clumping will be observed and it can concluded that the blood was of human origin.