
بسم الله الرحمن الرحيم

Forensic serology

Introduction

Forensic serology is the detection, classification and study of various bodily fluids such as :

- Blood
- Semen
- fecal matter
- perspiration, and
- their relationship to a crime scene.

A forensic serologist may also be involved in DNA analysis and bloodstain pattern analysis.

Serology

Serology – term used to describe a broad range of laboratory tests using reactions of blood serum and body fluid.

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

- blood typing.
- characterization of unknown blood.
- stain patterns for crime reconstruction.
- paternity testing.
- semen identification in rape cases.
- DNA techniques used for identification.

Blood terminology

ABO blood groups: based on having A, B, both or none of the factors on the red blood cell

Rh factor: may be present on the red blood cell; positive if present and negative if not.

Cont……..

Antigen: a substance found on a red blood cell

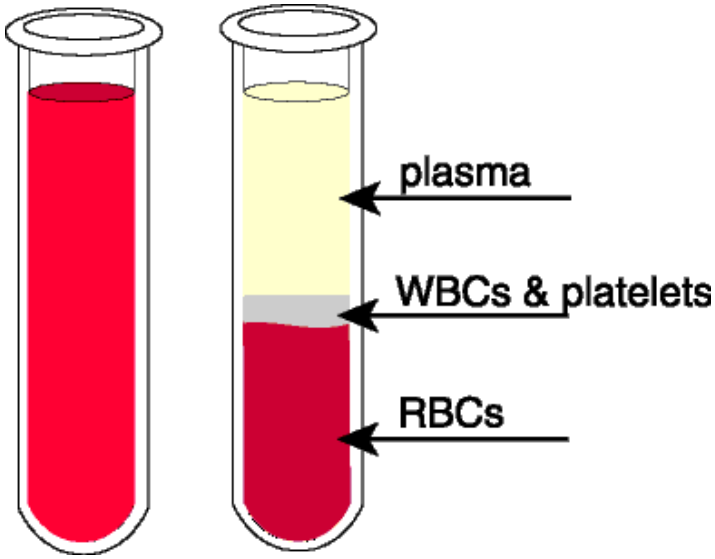
Antibody: a substance that reacts with an antigen

Agglutination: clumping of red blood cells; will result if blood types with different antigens are mixed.

Blood composition

Blood is slightly alkaline fluid which is mixture of many components:

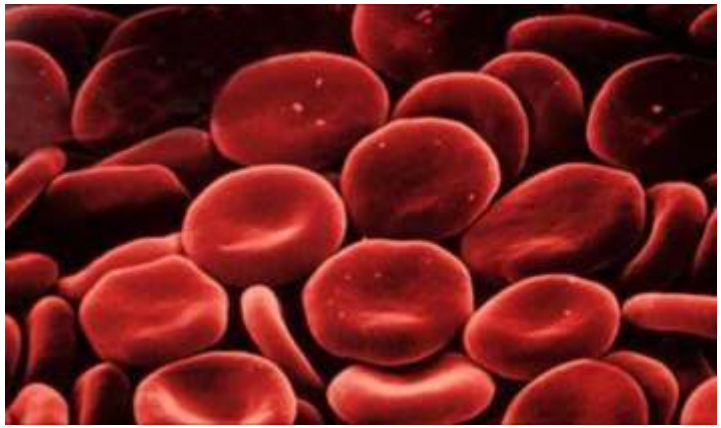
- Cells
- Inorganic substances (salts)
- Enzymes
- Water
- Proteins

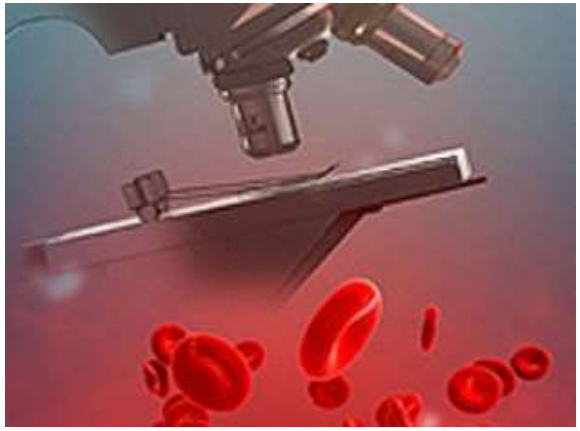


that circulates though out the vascular system carrying nourishment and transporting oxygen and waste

Blood cells

The most non-fluid portion of blood consists of red cells which out number white cells by five hundred to one.





While medical scientist are more interested in white cells, forensic scientist are more interested in red cells and secondly with serum.

Cont……..

- With serum the analyst can determine the freshness of blood sample because serum clots several minutes after exposure to air. (a centrifuge is necessary to separate clotted material from the rest of serum)

- In serum also found antibodies, which have important forensic implications.

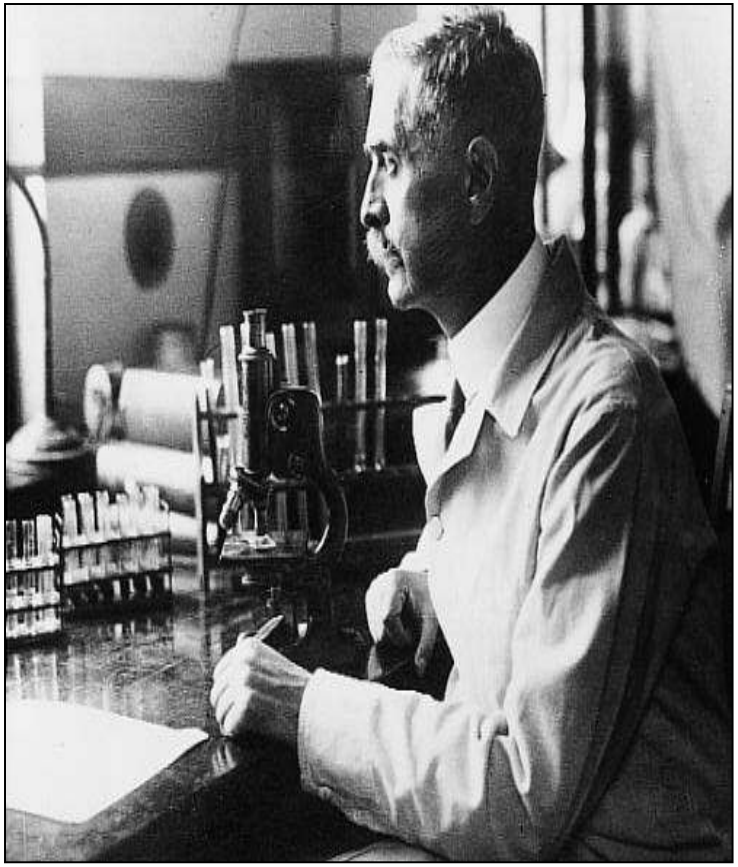
History

1901 KARL LANDSTEINER

First to identify ABO human blood groups

Nobel prize in 1930 for work

1937: identified Rh factor (+ or -)



Blood typing

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

The two most common blood typing systems used are the A-B-O method and the Rh method

- 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

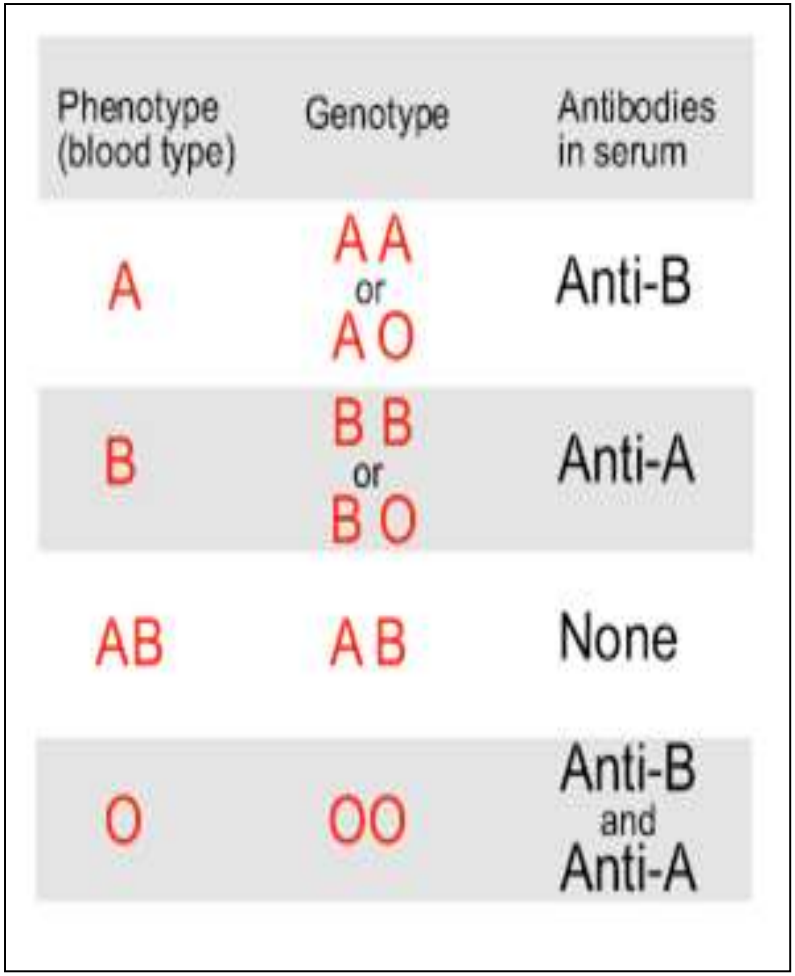
Cont……

The ABO Blood System

Blood Type (genotype)	Type A (AA, AO)	Type B (BB, BO)	Type AB (AB)	Type O (OO)
Red Blood Cell Surface Proteins (phenotype)	 A agglutinogens only	 B agglutinogens only	 A and B agglutinogens	 No agglutinogens
Plasma Antibodies (phenotype)	 b agglutinin only	 a agglutinin only	NONE. No agglutinin	 a and b agglutinin

Blood type basics

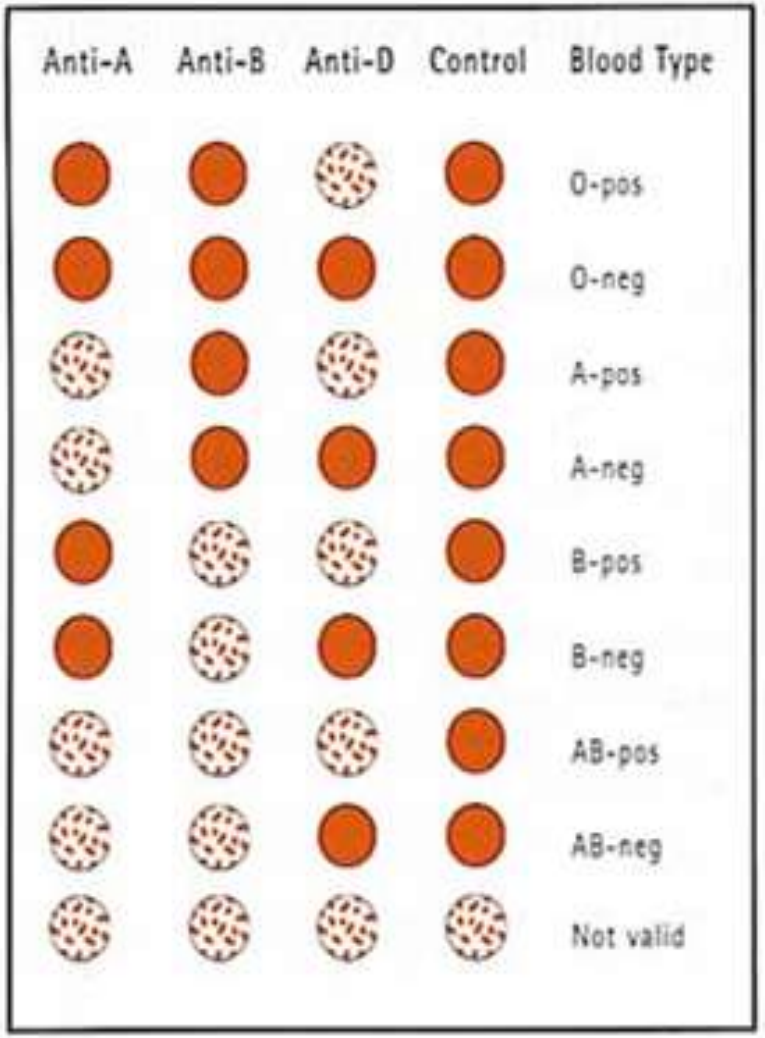
- Four blood types: A (39%), B (11%), AB (4%) and O (46%)
- Rh Factor: 83% of population are positive
- Testing for blood type is done through agglutination and antibodies



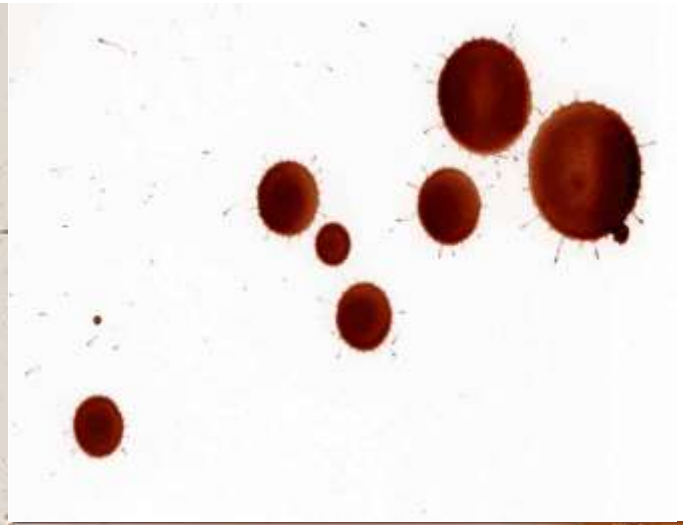
Phenotype (blood type)	Genotype	Antibodies in serum
A	A A or A O	Anti-B
B	B B or B O	Anti-A
AB	A B	None
O	O O	Anti-B and Anti-A

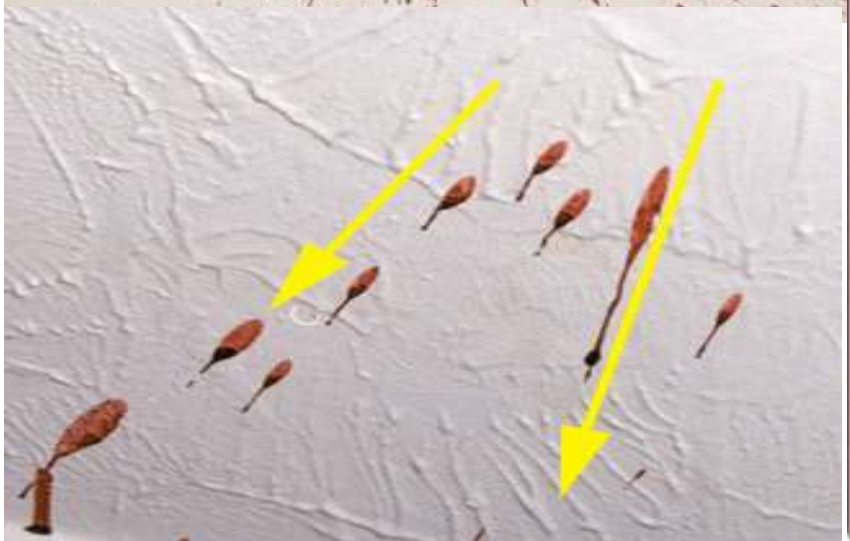
Blood typing

1. Separately add A and B antibodies to a few drops of the blood sample.
2. Look for agglutination.
3. Agglutination will occur only if the antigens are present on the RBC.
4. No agglutination with either = O.
5. Agglutination with both = AB



BLOODSTAIN PATTERN ANALYSIS





Introduction

Definition:

A field of forensic investigation that deals with the physical properties of blood and the patterns produced under different conditions as a result of different forces being applied to the blood, blood as a fluid follows the laws of physics.

Cont.....

- Bloodstain Pattern Analysis is the scientific study of bloodstains to assist in establishing spatial and sequential events occurring during and sometimes after the act of bloodshed.
- The diameter and shape of blood splatters, which reflect the origin and trajectory of external blood flow in the context of homicide or violent death, in which the skin surface is disrupted.

Cont……

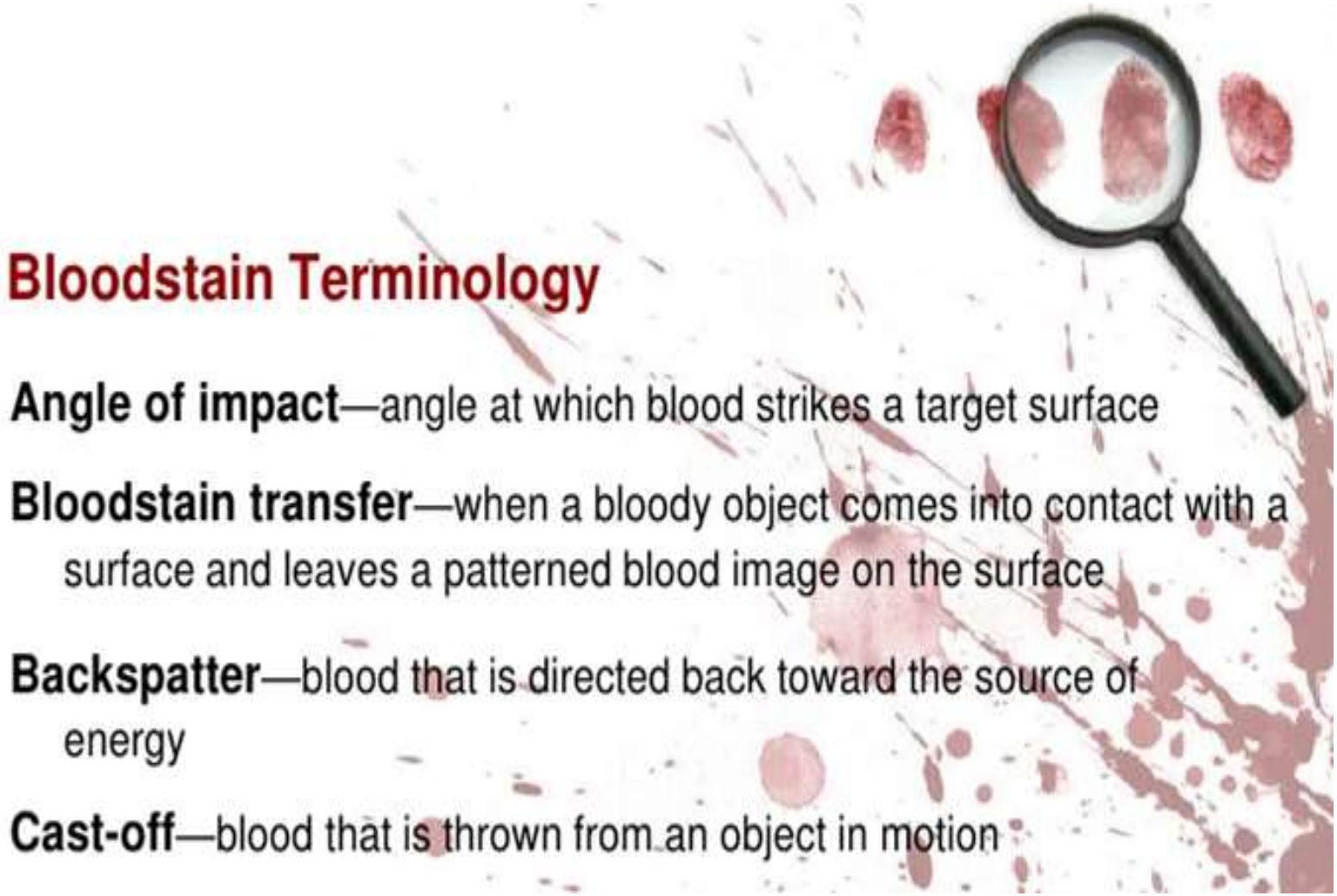
- The science of bloodstain pattern analysis applies scientific knowledge from other fields to solve practical problems. Such as:
- Biology
- Chemistry
- Maths &
- physics

Cont……..

Also known as:

- Blood spatter Pattern Analysis
- OR
- Bloodstain Pattern Investigation (BPA/BPI)
- Reconstructing events that must have happened to produce bleeding.
- Requires a BPA specialist.

Bloodstain terminology



Cont……

Contact stain—bloodstains caused by contact between a wet blood-bearing surface and a second surface that may or may not have blood on it

- **Transfer**—an image is recognizable and may be identifiable with a particular object
- **Swipe**—wet blood is transferred to a surface that did not have blood on it
- **Wipe**—a non-blood-bearing object moves through a wet bloodstain, altering the appearance of the original stain

Cont……..

Directionality—relates to the direction a drop of blood travels in space from its point of origin

Terminal velocity—the greatest speed to which a free-falling drop of blood can accelerate in air. It is dependent upon the acceleration of gravity and the friction of the air against the blood—approximately 25.1 feet/second.

- **High velocity**—greater than 25 feet per second, usually 100 feet per second; gives a fine mist appearance
- **Medium velocity**—5 to 25 feet per second
- **Low velocity**—5 feet per second or less

Blood pattern reconstruction

Scene pattern reconstruction

- Stain condition
- Pattern
- Distribution
- Location
- Directionality

Lab results reconstruction

- Genetic marker typing
- Age determination
- Source determination
- Race determination
- Sex determination

Blood stain evidence may reveal:

- Origin of blood stain.
- Distance of blood stain from target.
- Direction from which blood impacted.
- Speed with which blood left its source.
- Position of victim and assailant.
- Movement of victim and assailant.
- Number of blows and shots.

Liquid blood

- **Physical properties:**

1.Viscosity

2.Surface tension

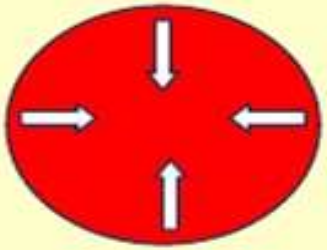
3.Specific gravity

- **Shows projectile motion**

Cont……..

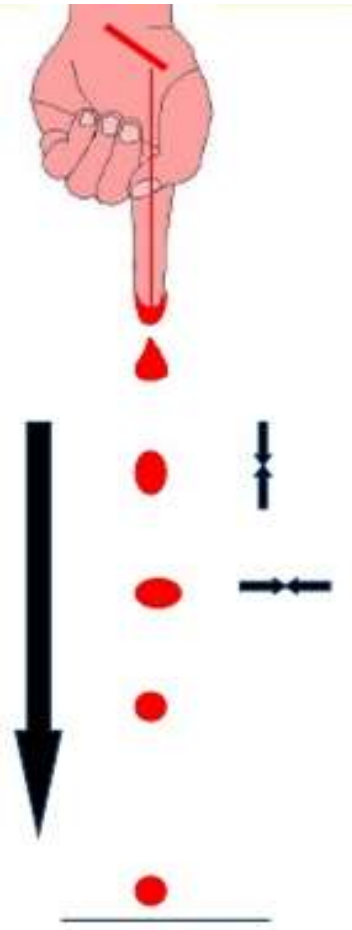
Surface Tension

- Resistance to penetration & separation
- Surface acts to reduce surface area



- Smallest SA to Volume ratio is offered by sphere

Cont……



Dripping Blood

Blood trickles downwards

Blood drop grows until Wt (G) > S.T.

Single drop breaks free (teardrop shape)

Surface tension pulls in vertically

And horizontally

Shape settles into sphere (0.05 ml)

Does not break up until impact

Characteristics of blood drop

- A blood droplet remains spherical in space until it collides with a surface.
- Once a blood droplet impacts a surface, a blood stain is formed.
- Droplets falling from different height, with different angle, hitting the same surface, will produce stains with different pattern and shape.

Conditions affecting shape of blood droplet

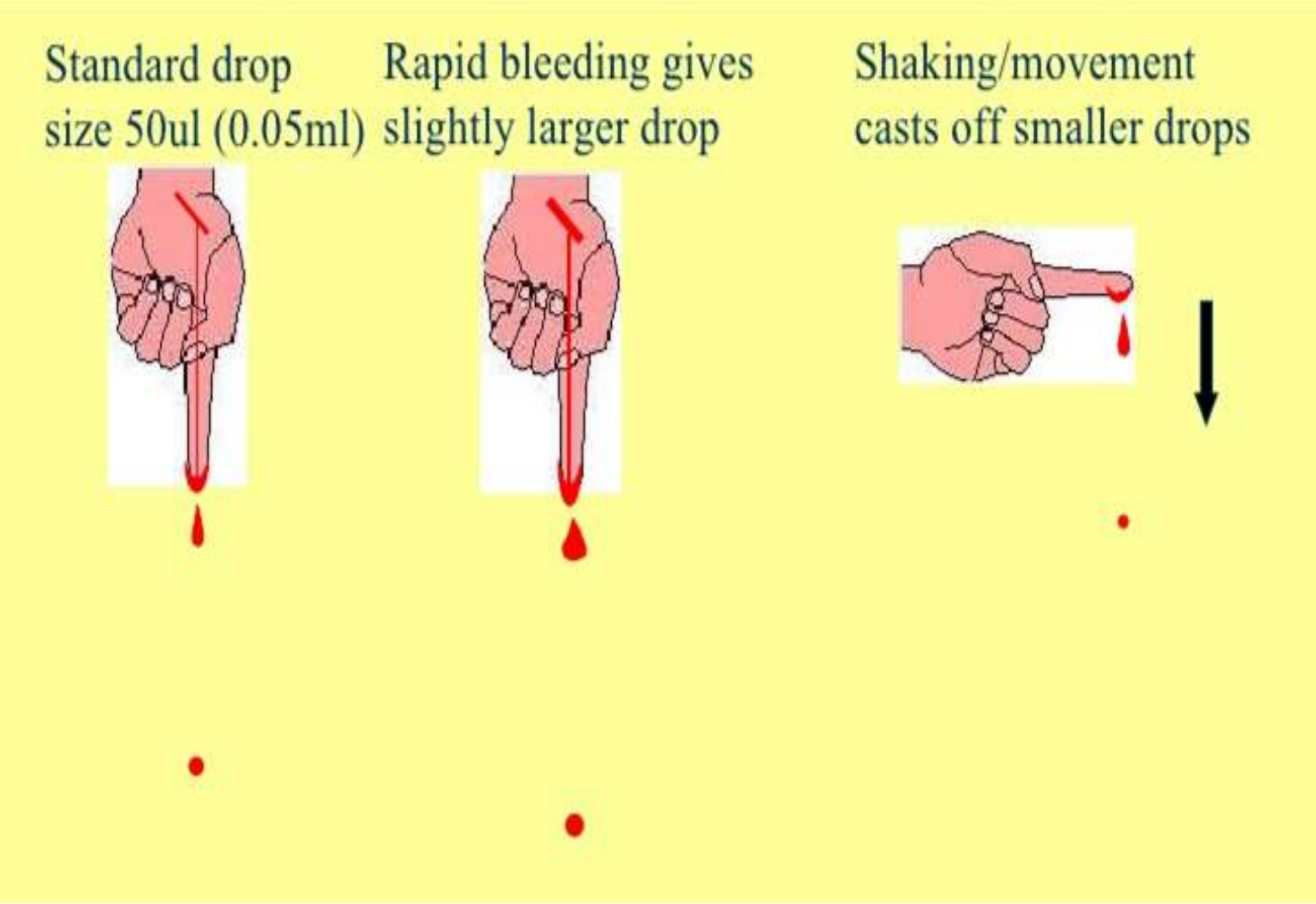
Shape and size of blood spot

- **Depends mostly on nature of target surface:**
  - Texture (rough or smooth)
  - Porous or non porous

- **Size is related to distance fallen:**
  - Standard 50ul drop of blood

- **There is a little change in spot diameter beyond a fall distance of 1.2 m.**

1. Size of blood drop

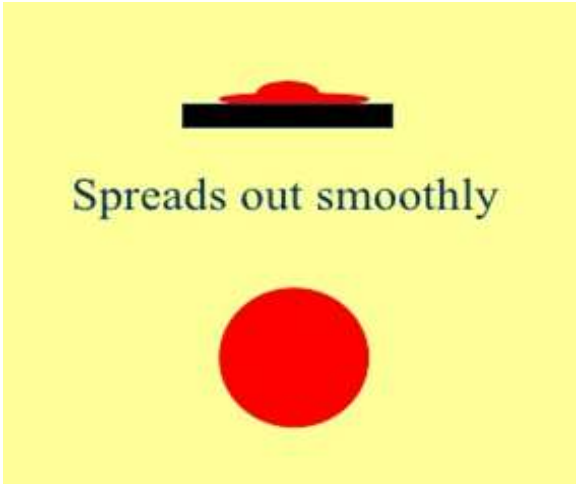


2. Target surface

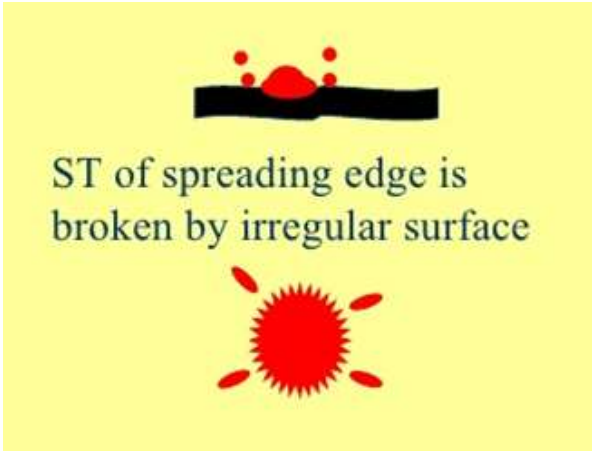
- The harder and less porous the surface, the less the blood drop will break.
- The softer and more porous the surface, the more the blood drop will break apart.
- The pointed end of blood stain shows the direction of travel.

Cont……..

- Smooth surface:



- Rough surface:



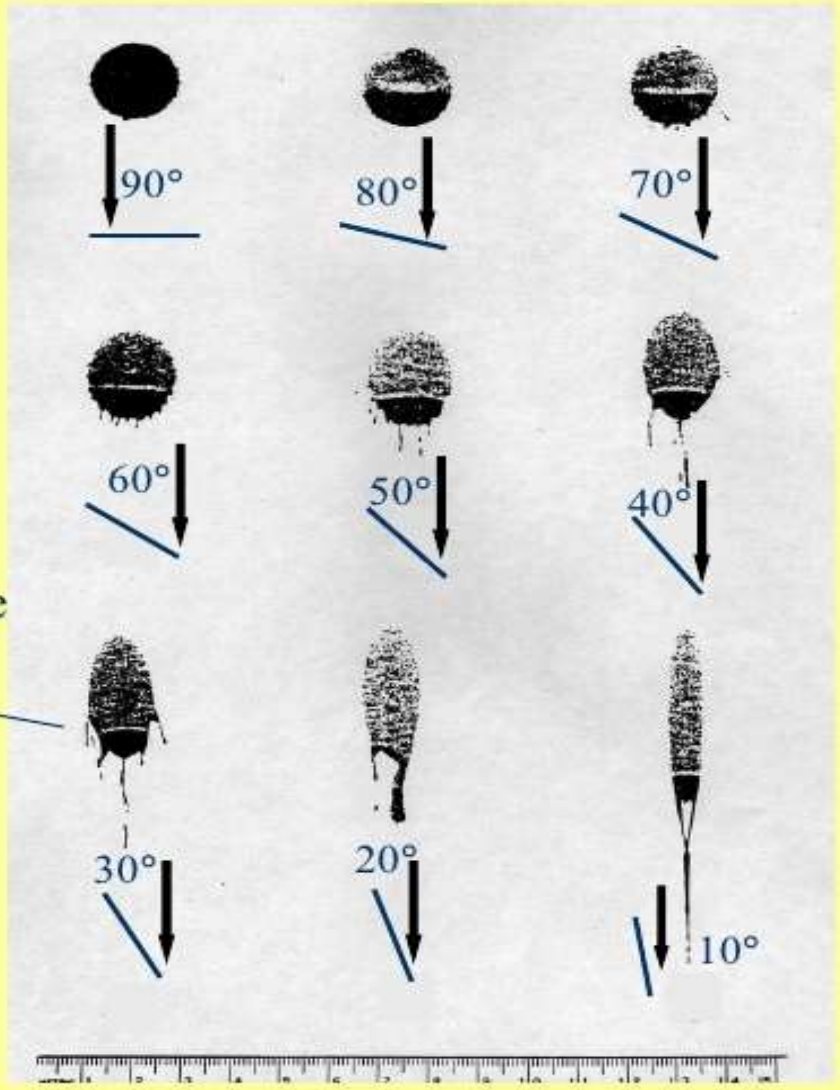
3. Angle of impact

- **The more acute the angle of blood, the more elongated stain.**
- 90° angle drops are perfectly round drops.
- 80° are more elliptical shape.
- At about 30° the stain will begin to produce a tail.
- **The more acute the angle, the easier to determine the direction of travel.**

Cont……

14

Angle of Impact



Gravitational dense zone at lower edge

Adapted from
Introduction to Forensic Sciences,
W. Eckert, CRC, 1997

Wet vs. dry blood

- Wet blood is more significant than dry blood because scientist can perform more tests in order to investigate exact crime.
- For example alcohol and drug content can be determined from wet blood only.
- Blood is dried after 3-5 mins.
- Color changes from deep red to brown to black.
- Blood can be categorized into pools, drops, smear or crusts.

Blood testing by using different techniques

Characterization of bloodstain

1.Is it blood?

2.Which species it come from?

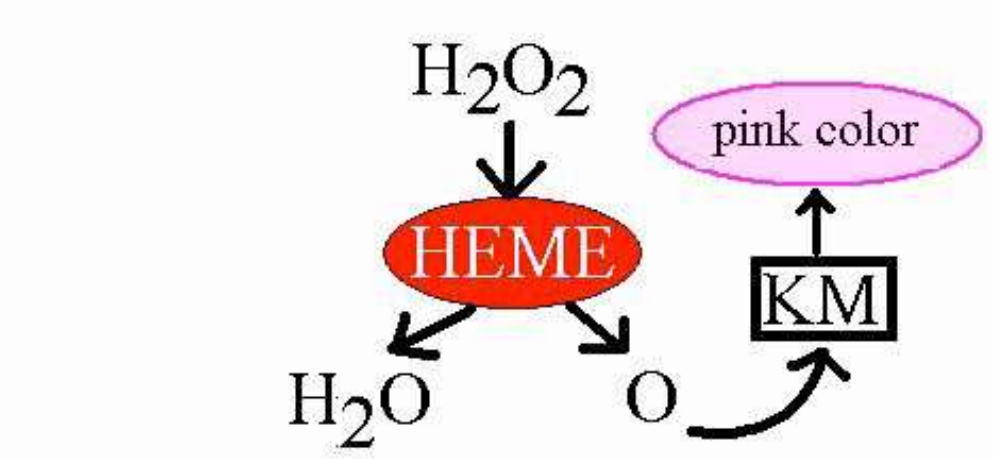
3.If it's human, can it be associated with a particular individual?

4.Can the sex, age and race of the source of blood be determined?

1. Blood or not?

- To determine whether or not blood is present at a crime scene investigators color and crystalline tests are used.
- Firstly, ***benzidine test*** was used (carcinogenic). Benzidine +blood stain +hydrogen peroxide= pink color

- Now ***Kastle-Meyer test*** is used: phenolphthalien+bloodstain+hydrogen peroxide= bright pink color.



- **Microcrystalline Test**
- Haem forms crystals when reacted with certain reagents. The most common such reagent is pyridine, which forms characteristic pink crystals. The test is carried out on a microscope slide, with the reagents being added to the stain under a cover slip, and crystal formation observed microscopically.

Luminol test (Invisible blood stains)

- Luminol, a chemical sprayed on carpets and furniture, reveals a slightly flourescent light in the dark where bloodstains are present.
- Long dried blood has tendency to crystallize or made to crystallize with various chemicals.
- Luminol is made up in alkaline solution (pH 10.4-10.8) using sodium carbonate, and sodium perborate ($NaBO_3.H_2O$).
- The solution is applied as a spray and the presence of blood produces a bluish luminescence which persists for about 45 seconds.


Luminol test

Cont.....

Instrumental tests:

Chromatographic techniques.

Tests are used practically for several different purposes including:

1. Confirmation of the nature of visible stains.
2. The detection of non-visible stains. and
3. The enhancement of hard to see stains.

Cont.....

- **High performance liquid chromatography (HPLC)** can be used to confirm the identity of blood using the absorbance of haemoglobin for detection. This method can also be used to identify the species of origin from variations in the globin chains, to distinguish foetal haemoglobin from adult haemoglobin, and to give an estimate of the age of a bloodstain.

2. Animal or human blood?

Precipitation test:

- This test involves injecting an animal , usually a rabbit , with human blood.
- Animal's body produces human antibodies.
- Extracted from animal's serum.
- Antiserum then placed a sample from crime scene.
- Clotting reveals that blood source is human.

Cont.....

- **Gel Diffusion**
- Human antibodies and bloodstain are placed in wells on an agar gel.
- If antibodies and antigens move towards each other and form a line of precipitation, it is human blood.
- **Electrophoretic Method**
- Similar to Gel diffusion except electrical current is used to move antibodies and antigens towards each other

3.Particular individual?

- To test a blood sample, for determining a particular individual forensic investigator must have adequate and quality blood sample.
- Tests:
- Blood group testing
- DNA finger printing etc.

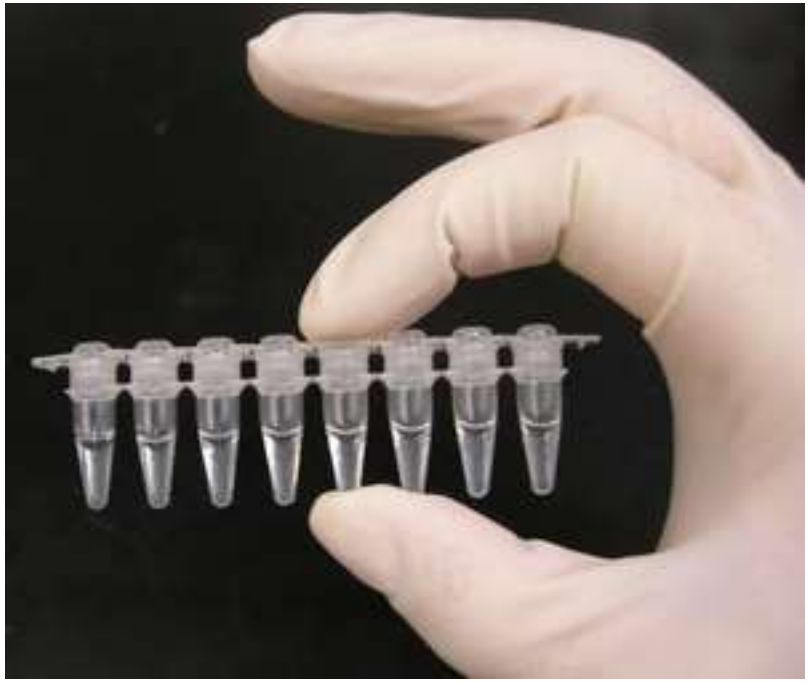
4. Age, sex and race?

- various color and nitrate tests, and heredity principles are applied.
- No exact determination possible.
- **Age determination:** Clotting and crystallization of blood.
- **Sex determination:** testosterone and chromosome testing.
- **Race determination:** racial genetic markers involving protein and enzyme tests.

Forensic DNA analysis

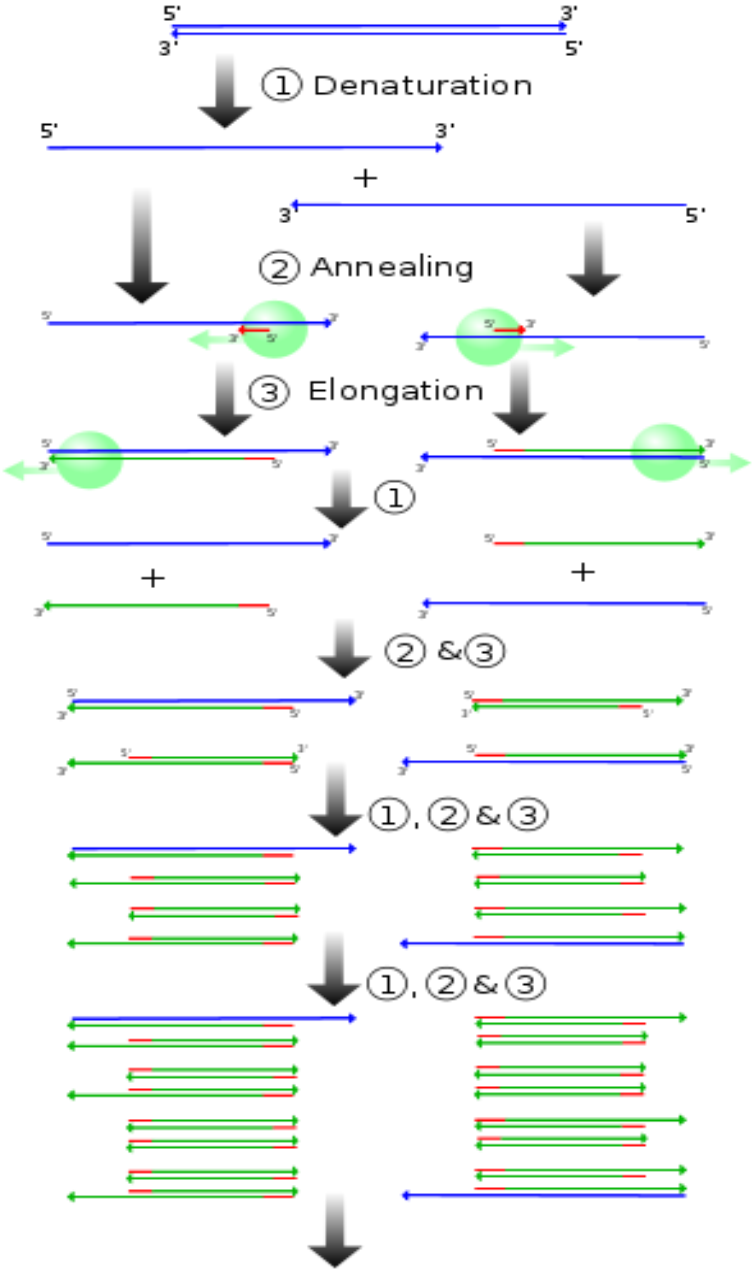
PCR and RFLP

Polymerase chain reaction



- The **polymerase chain reaction** (**PCR**) is a biochemical technology in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.
- As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the DNA template is exponentially amplified.

- A basic PCR set up requires several components and reagents. These components include:
- *DNA template* that contains the DNA region (target) to be amplified.
- Two *primers* that are complementary to the 3' (three prime) ends of each of the sense and anti-sense strand of the DNA target.
- *Taq polymerase* or another DNA polymerase with a temperature optimum at around 70 °C.
- *Deoxynucleoside triphosphates* (dNTPs, sometimes called "deoxynucleotide triphosphates"; nucleotides containing triphosphate groups), the building-blocks from which the DNA polymerase synthesizes a new DNA strand.
- *Buffer solution*, providing a suitable chemical environment for optimum activity and stability of the DNA polymerase.
- *Divalentcations* magnesium or manganese ions; generally Mg^{2+} is used, but Mn^{2+} can be utilized for PCR-mediated DNA mutagenesis, as higher Mn^{2+} concentration increases the error rate during DNA synthesis



Exponential growth of short product

- *Initialization step*: This step consists of heating the reaction to a temperature of 94–96 °C (or 98 °C if extremely thermostable polymerases are used), which is held for 1–9 minutes. It is only required for DNA polymerases that require heat activation by hot-start PCR.

- *Denaturation step*: This step is the first regular cycling event and consists of heating the reaction to 94–98 °C for 20–30 seconds. It causes DNA melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.

- *Annealing step*: The reaction temperature is lowered to 50–65 °C for 20–40 seconds allowing annealing of the primers to the single-stranded DNA template. Typically the annealing temperature is about 3-5 degrees Celsius below the Tm of the primers used. Stable DNA-DNA hydrogen bonds are only formed when the primer sequence very closely matches the template sequence. The polymerase binds to the primer-template hybrid and begins DNA formation.

- *Extension/elongation step*: The temperature at this step depends on the DNA polymerase used; Taq polymerase has its optimum activity temperature at 75–80 °C, and commonly a temperature of 72 °C is used with this enzyme. At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs that are complementary to the template in 5' to 3' direction, condensing the 5'-phosphate group of the dNTPs with the 3'-hydroxylgroup at the end of the nascent (extending) DNA strand. The extension time depends both on the DNA polymerase used and on the length of the DNA fragment to be amplified. As a rule-of-thumb, at its optimum temperature, the DNA polymerase will polymerize a thousand bases per minute. Under optimum conditions, i.e., if there are no limitations due to limiting substrates or reagents, at each extension step, the amount of DNA target is doubled, leading to exponential (geometric) amplification of the specific DNA fragment.

- *Final elongation*: This single step is occasionally performed at a temperature of 70–74 °C for 5–15 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended.

- *Final hold*: This step at 4–15 °C for an indefinite time may be employed for short-term storage of the reaction.

Reverse transcriptase PCR (RT-PCR)

- This PCR was designed to amplify RNA sequences (especially mRNA) through synthesis of cDNA by reverse transcriptase (RT).
- Subsequently, this cDNA is amplified using PCR.
- This type of PCR has been useful for diagnosis of RNA viruses, as well as for evaluation of antimicrobial therapy

Real time PCR

- To quantify the number of copies of nucleic acids during PCR
- Intercalating agents such as SYBR Green are fluorochromes dramatically increase the fluorescence by binding to a double-stranded DNA .
- Thus, the increase of DNA in each cycle reflects a proportional increase in the emitted fluorescence.

Multiplex PCR

- It is a modification of polymerase chain reaction in order to rapidly detect deletions or duplications in a large gene.
- This process amplifies genomic DNA samples using multiple primers and a temperature-mediated DNA polymerase in a thermal cycler

Nested PCR:

- Increases the specificity of DNA amplification, by reducing background due to non-specific amplification of DNA. Two sets (instead of one pair) of primers are used in two successive PCRs. Nested PCR is often more successful in specifically amplifying long DNA fragments than conventional PCR, but it requires more detailed knowledge of the target sequences

Hot-start PCR:

- A technique that reduces non-specific amplification during the initial set up stages of the PCR. It may be performed manually by heating the reaction components to the melting temperature (e.g., 95°C) before adding the polymerase. Hot-start/cold-finish PCR is achieved with new hybrid polymerases that are inactive at ambient temperature and are instantly activated at elongation temperature.

Colony PCR

- the screening of bacterial (E.Coli) or yeast clones for correct ligation or plasmid products. Selected colonies of bacteria or yeast are picked with a sterile toothpick or pipette tip from a growth (agarose) plate. This is then inserted into the PCR master mix or pre-inserted into autoclaved water. PCR is then conducted to determine if the colony contains the DNA fragment or plasmid of interest

Allele-specific PCR:

- A diagnostic or cloning technique which is based on single-nucleotide polymorphisms (SNPs) (single-base differences in DNA). It requires prior knowledge of a DNA sequence, including differences between alleles, and uses primers whose 3' ends encompass the SNP. PCR amplification under stringent conditions is much less efficient in the presence of a mismatch between template and primer, so successful amplification with an SNP-specific primer signals presence of the specific SNP in a sequence.

Application of PCR in forensics

- Genetic basis of diseases with sudden death can be investigated.
- Forensic molecular pathology involves application of molecular biology in medical science to investigate the genetic basis of
- pathophysiology of diseases that lead to deaths ("molecular autopsy").

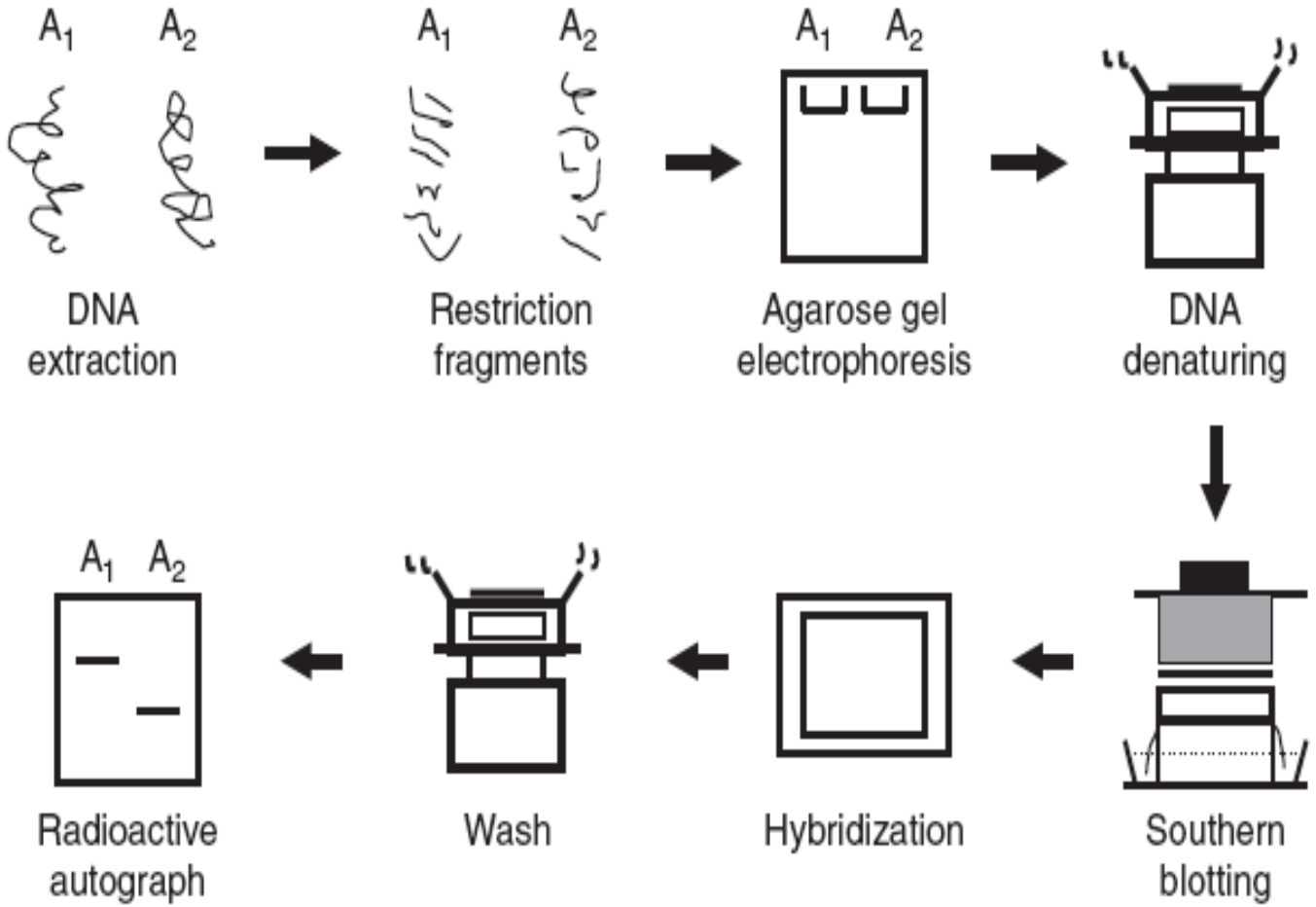
Cont……..

- To establish the filiations of a person
- Paternity testing
- To obtain evidence from minimal samples of saliva, semen or other tissue debris

RFLP

- The DNA sample is broken into pieces (digested) by restriction enzymes and the resulting *restriction fragments* are separated according to their lengths by gel electrophoresis.
- In addition to genetic fingerprinting RFLP was an important tool in genome mapping, localization of genes for genetic disorders, determination of risk for disease, and paternity testing

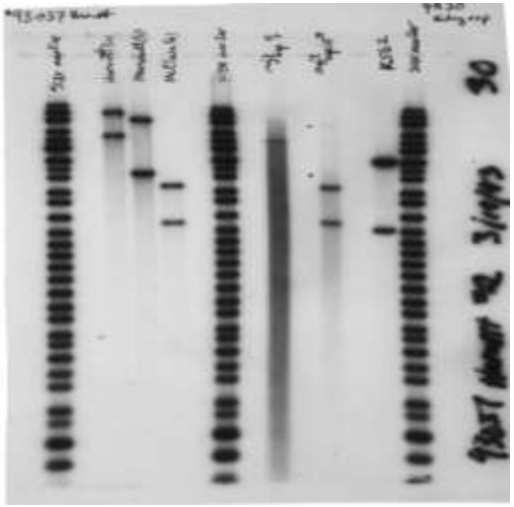
Cont……


A1
A2
DNA extraction
Restriction fragments
Agarose gel electrophoresis
DNA denaturing
Southern blotting
Hybridization
Wash
Radioactive autograph

FORENSIC DNA ANALYSIS

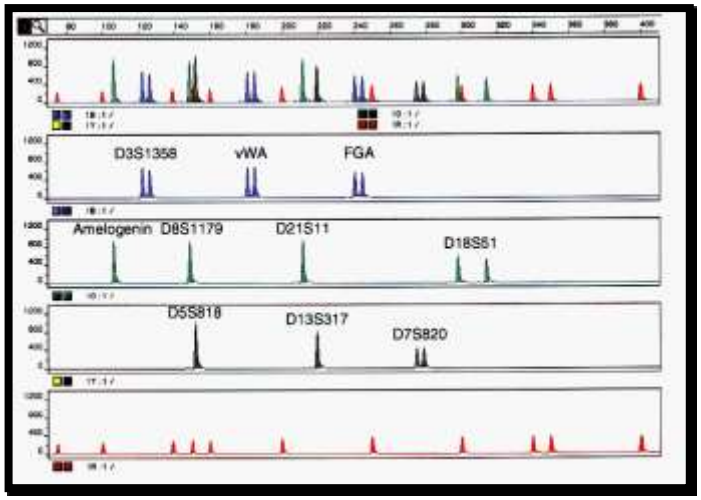
Short Tandem Repeats (STRs)

Three generations of DNA testing



RFLP
AUTORAD
Allele = BAND

DQ-alpha
TEST STRIP
Allele = BLUE



Automated STR
ELECTROPHEROGRAM
Allele = PEAK

STRs

- **Short tandem repeats**
- **Describes a type of DNA polymorphism in which:**
  - **a DNA sequence repeats**
  - **over and over again**
  - **and has a short (usually 4 base pair) repeat unit**
- **A length polymorphism -- alleles differ in their length**

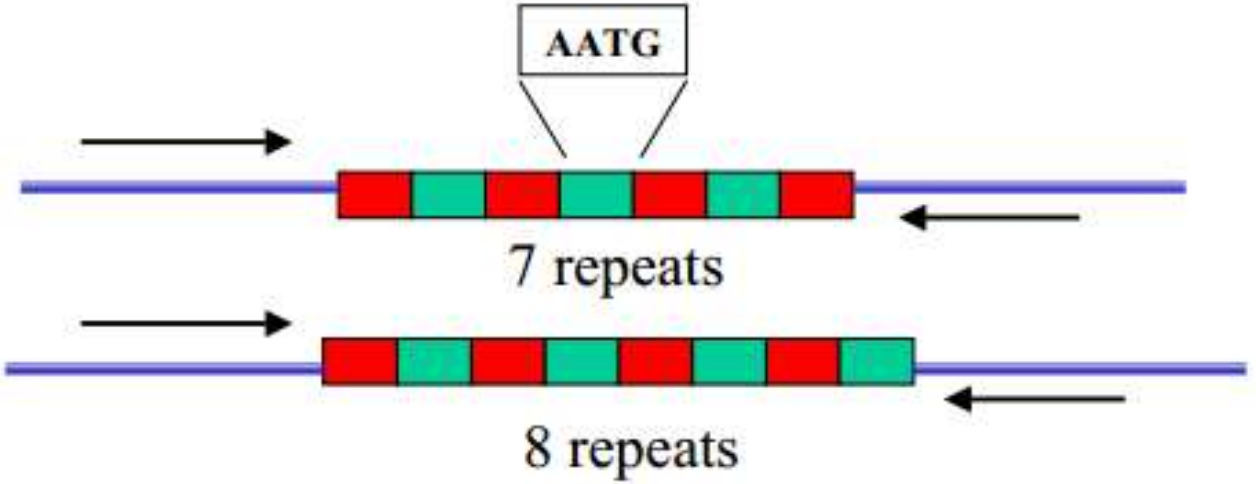
3 repeats: AATG AATG AATG

4 repeats: AATG AATG AATG AATG

5 repeats: AATG AATG AATG AATG AATG

6 repeats: AATG AATG AATG AATG AATG AATG

Short Tandem Repeats (STRs)



the repeat region is variable between samples while the flanking regions where PCR primers bind are constant

Homozygote = both alleles are the same length

Heterozygote = alleles differ and can be resolved from one another

Application in forensics

- Individual identification possible
- People differ in length at these loci
- Genetic fingerprinting
- Paternity, maternity analysis

Person 1 ..GCCAGCTAGCTAGCTAGCTAGCTAGCTTTCAT..

Person 2 ..GCCAGCTAGCTAGCTAGCTAGCTTTCAT..

Person 3 ..GCCAGCTAGCTAGCTAGCTAGCTAGCTAGCTT..

1 2 3 4 5 6 7

Steps to develop SSR markers

- Construct small-insert clone library
- Screen it by hybridizing labelled oligo (with SSR

motif of interest)

- Sequence positive clone

Design primers in single copy regions flanking SSR repeats such that the amplified fragmentswill be > 50 bp and < 350 bp

- Identify size polymorphism on PAGE gels.

Automated STR Test

Basic steps in analysis

- **Extraction:**
- Separates DNA from sample
- **Amplification or PCR:**
- Amplifies small portions of DNA (STR regions)
- **Separation:**
- Separates amplified fragments according to size.

Crime Scene Samples & Reference Samples

- Extract and purify DNA

Differential extraction in sex assault cases separates out DNA from sperm cells

Extract and Purify DNA

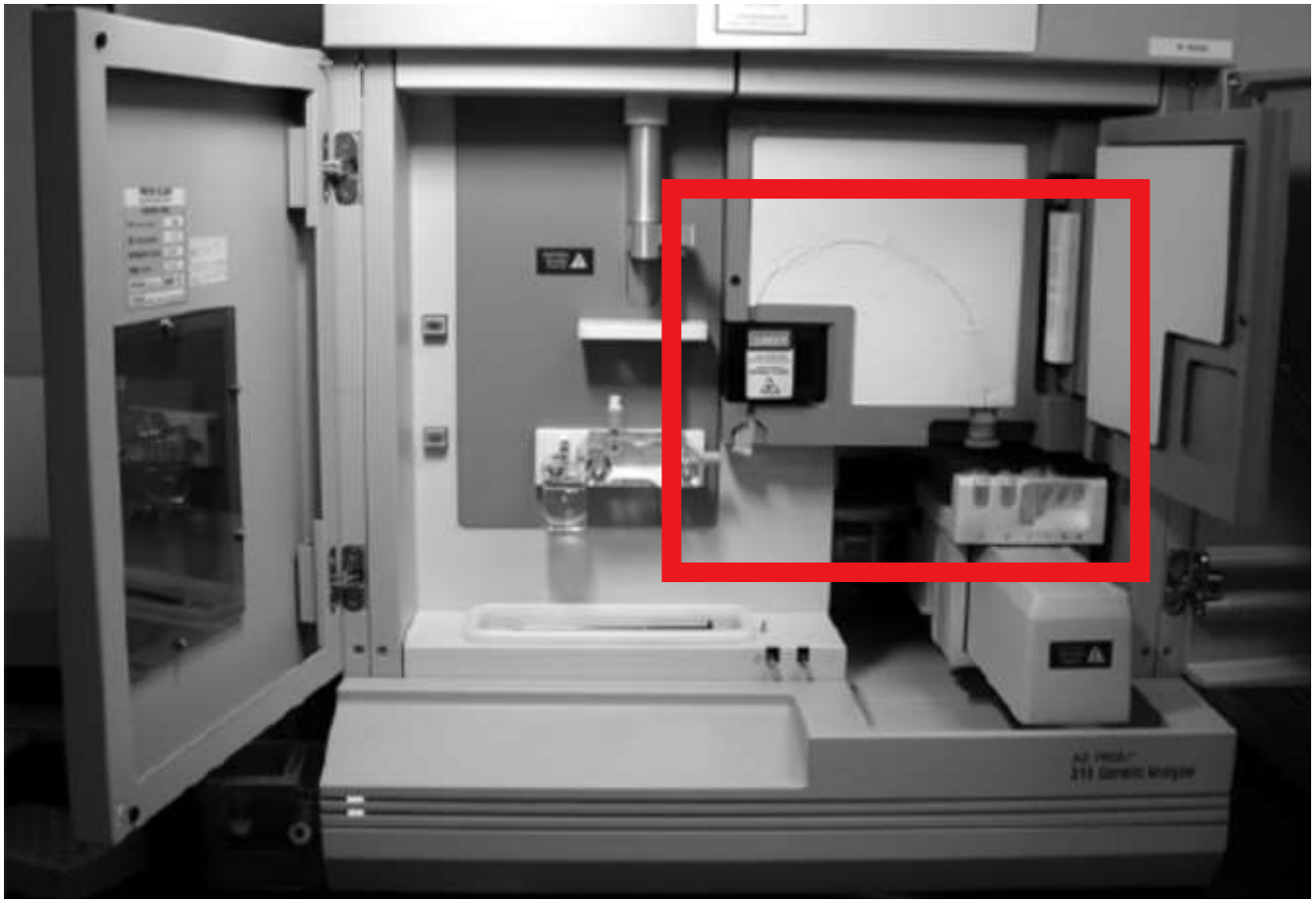
- Add primers and other reagents

PCR Amplification

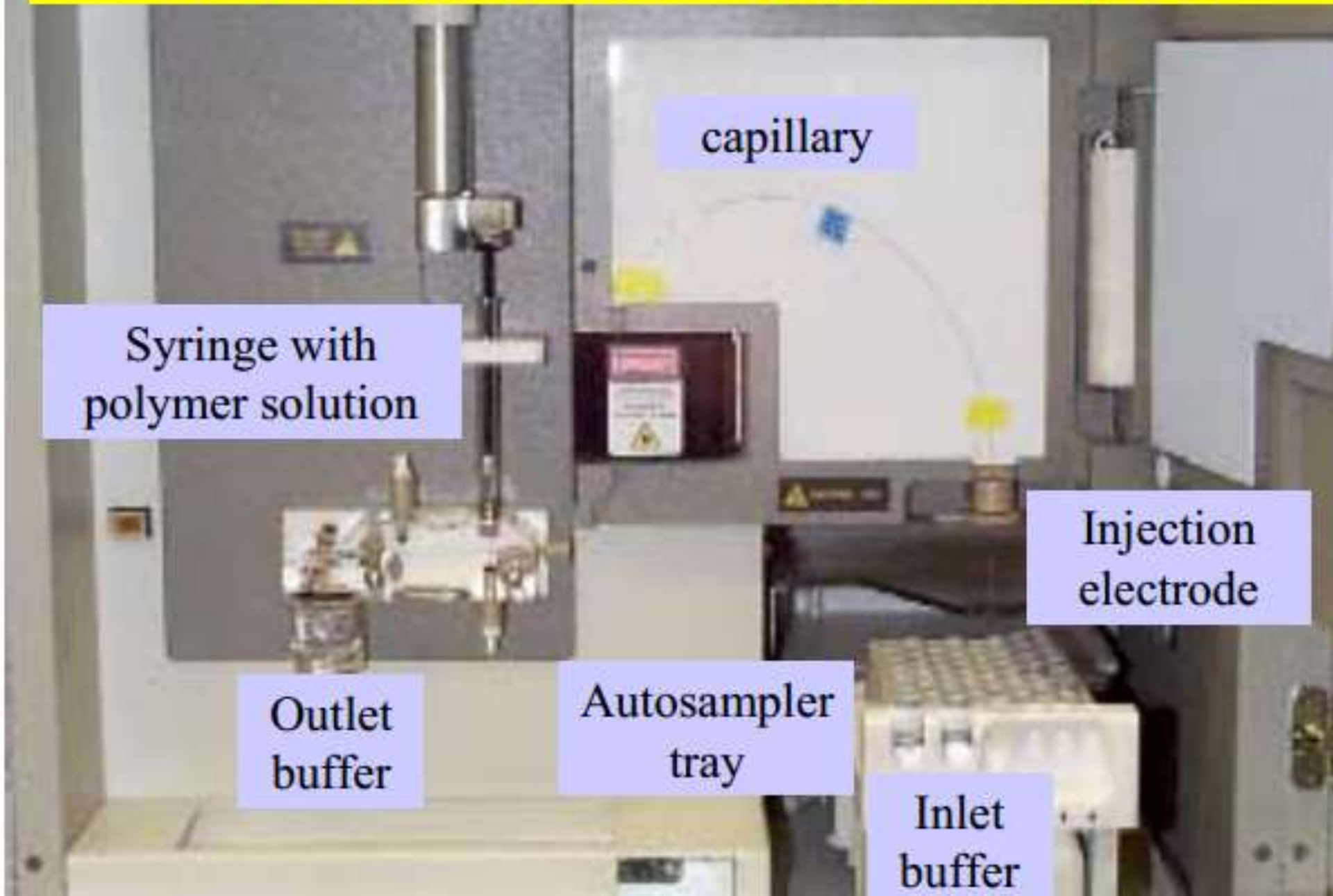
- DNA regions flanked by primers are amplified

Groups of amplified STR products are labeled with different colored dyes
(blue, green, yellow)

The ABI 310 Genetic Analyzer: SIZE, COLOR & AMOUNT

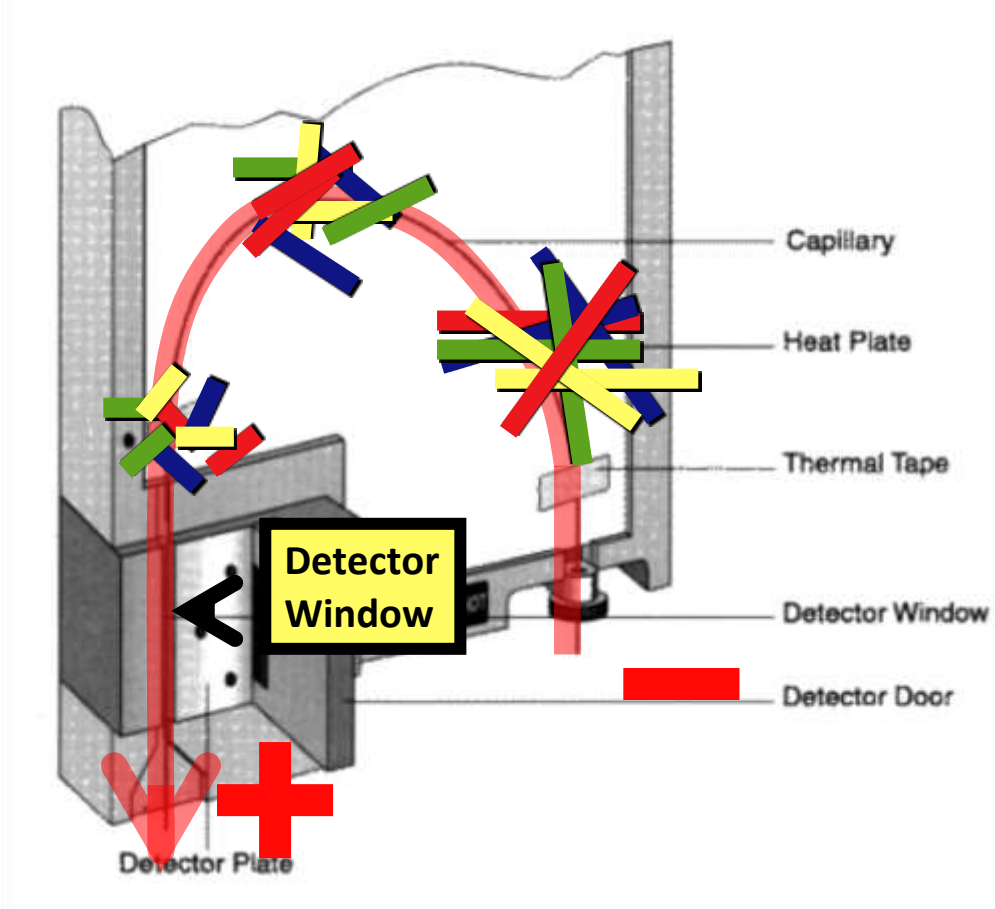


ABI Prism 310 Genetic Analyzer



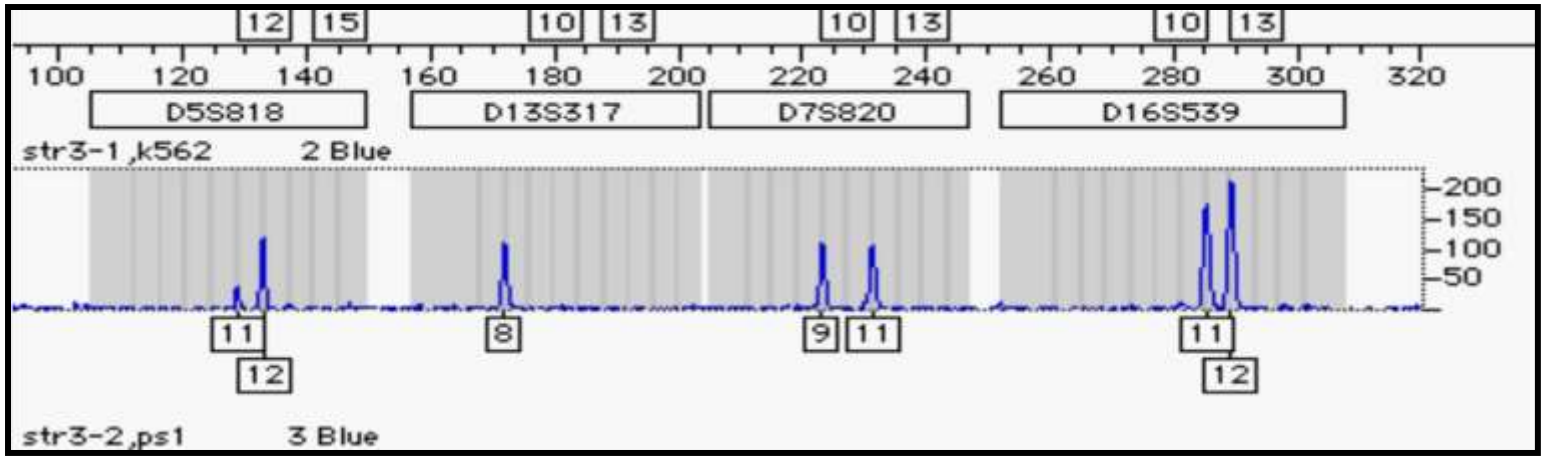
ABI 310 Genetic Analyzer: Capillary Electrophoresis

- **Amplified STR DNA injected onto column**
- **Electric current applied**
- **DNA pulled towards the positive electrode**
- **DNA separated out by size**
  - **Large STRs travel slower**
  - **Small STRs travel faster**
- **Color of STR detected and recorded as it passes the detector**



Capillary Electrophoresis

Sample will have one or two peaks at each loci.



Resources

- Books
  - **'Forensic DNA Typing' by John M. Butler (Academic Press)**
- Internet
  - **Applied Biosystems Website: http://www.appliedbiosystems.com/ (see human identity and forensics)**
  - **Forensic Bioinformatics Website: http://www.bioforensics.com/**
  - **STR base: http://www.cstl.nist.gov/biotech/strbase/ (very useful)**
- Scientists
  - **Larry Mueller (UC Irvine)**
  - **Simon Ford (Lexigen, Inc. San Francisco, CA)**
  - **William C. Thompson (UC Irvine)**
  - **William Shields (SUNY, Syracuse, NY)**
  - **Marc Taylor (Technical Associates, Ventura, CA)**
  - **Keith Inman (Forensic Analytical, Haywood, CA)**
- Testing laboratories
  - **Technical Associates (Ventura, CA)**
  - **Indiana State Police (Indianapolis, IN)**
- Other resources
  - **Forensic Bioinformatics (Dayton, OH)**