DIRECTORATE OF DISTANCE & CONTINUING EDUCATION
MANONMANIAM SUNDARANAR UNIVERSITY
TIRUNELVELI- 627 012

OPEN AND DISTANCE LEARNING (ODL) PROGRAMMES
(FOR THOSE WHO JOINED THE PROGRAMMES FROM THE ACADEMIC YEAR 2023–2024)

M. Sc. Chemistry
Course Material

Skill Enhancement Course - II
Forensic Chemistry
Course Code: SCHS31

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Skill Enhancement Course
FORENSIC CHEMISTRY

UNIT I: ELEMENTARY FORENSIC SCIENCE

Definition of Forensic science, The role of Forensic laboratory, Biometrics in Personal Identification- Introduction, Concepts of Biometric Authentication, Role in person Identification - Face Recognition, IRIS, Retina Geometry, Hand Geometry, Speaker Recognition, Signature Verification.

UNIT II: FINGER PRINTING AND FORENSIC SEROLOGY

Fingerprinting - General principles of Finger Printing, Fingerprint Detection - **Powder tests:** – dry powder method, detection using cellophane tape- **Chemical tests:** – silver nitrate test, iodine fuming, ninhydrin, superglue (cyanoacrylate)and ruthenium oxide tests. Forensic Serology – Blood types, Characterization of Blood stains, Blood stains patterns. Testing of Saliva.

UNIT III: FORENSIC ANALYSIS

Forensic Drug Analysis: How drugs work - analysis of selected drug classes –Gamma hydroxybutyric acid (GHB), Gamma butyro lactone (GBL), Marijuana, Anabolic steroids, Heroin, Cocaine, Amphetamines.
Forensic analysis of Inks and paints: Questioned documents – Physical analysis, chemical analysis of inks and paper – analytical methods – Optical microscopy, fluorescent techniques, TLC, FT-IR.

UNIT IV: FORENSIC TOXICOLOGY

Forensic Toxicology: Overview - Sample types – Blood and Plasma, Urine, Vitreous fluid, Hair. Types of Forensic Toxicology – Alcohol, Postmortem toxicology, Sport Toxicology. Analytical methods in Forensic Toxicology – Breath alcohol test (BrAC). An introduction to DNA, Forensic DNA typing - methods of DNA typing - RFLP and PCR methods – Procedures for DNA typing, Applications of DNA testing.

UNIT V : CYBER CRIME TECHNOLOGY AND FORENSIC SCIENCE

Use of computers in Forensic science: Forensic Databases, Image Databases, DNA Database. Forensic Archiving of X-Ray Spectra, Video Image Processing and Animation Software, Use of Networks in Forensic Science.
Computer related crime: Definitions and types - Framework for Investigating Computer-Related Crime, Human Aspects of Computer Related Crime.

Recommended Text

1. Anil K. Jain, Arun A. Ross and Karthik Nandakumar, *Introduction to Biometrics*, Springer, 2011.
2. David E. Newton, *Forensic Chemistry*, Fact on File, Inc, 2007.
3. Suzanne Bell, *Forensic Chemistry,* Pearson International, Second Edition, 2014.
4. Stuart H. James and Jon J. Nordby, *Forensic Science - An Introduction to Scientific and Investigative Techniques,* CRC Press, 2003.

Reference Books

1. Saferstein R, Criminalistics: An introduction to Forensic Science: Prentice Hall, Eaglewood cliffs, New Jersey, 2001.
2. Editor – G.R. Sinha, *Advances in Biometrics - Modern Methods and Implementation Strategies*, Springer, 2019.
3. Editor – Jay A. Siegel, *Forensic Chemistry -Fundamentals and applications*, Wiley-Blackwell, First edition, 2016**.**
4. Max M. Houck**,** *Forensic Science-Modern methods of solving crime*, Praeger Publishers, 2007.
5. Kelly M. Elkins, *Introduction to Forensic chemistry***,** CRC Press, 2019.
6. Matthew Johll, *Investigating Chemistry: A Forensic Science Perspective*, W.H. Freeman & Co, Second Edition, 2008

Unit – 1

Elementary Forensic Science

Definition of Forensic Science

Forensic science can be defined as the application of science to law. As crime has become more prevalent in our society, law enforcement has found it increasingly necessary to seek assistance from the scientific community for advice and technical support. Much of this support has been in the field of forensic science. Science has come to occupy an important place in the field of criminal investigation. Criminal investigation can be defined as an inquiry into a particular crime. Because science plays such an important role, forensic science supplies information that is accurate and objective. In other words, we could say that forensic science is the application of science to criminal and civil laws that are enforced by the criminal justice system.

Forensic science exhibits an imperative responsibility in criminal interrogations, enabling law implementation to not just recognize possible culprits but also establish when and how a misdeed takes shape. The term 'forensic,' symbolizing a link to the legal sphere, accentuates that forensic science administers scientific tenets to matters of law. The role of forensic science is extensive, incorporating assorted territories such as DNA assessment, fingerprint analysis, autopsies, pathology, and toxicology. These areas jointly provide to untangling the cause of death and initiating potential interconnections to the accused.

The term criminalistics is often used in place of the term forensics. Criminalistics can be defined as a professional and scientific discipline which involves the recognition, identification, individualization, and analysis of physical evidence by application of the natural sciences in criminal matters. Criminology, is not the same as criminalistics. It is the scientific study of crime and the criminal. The crime scene investigator, when looking for a motive, will begin to shift from criminalistics to criminology.

The role of Forensic Laboratory

- Providing professional and technical assistance to the investigating officers to recognize appropriate evidence materials at the scene of crime, followed by proper

collection and preservations of the said materials so recognized, and dispatch of the relevant materials to the laboratory for examination

- Preliminary analysis of the clue materials at the scene of crime and thereafter forensic analysis of the clue materials by latest analytical methodologies in the laboratory
- Interpretation of the forensic results so obtained
- Deposing the material evidence so obtained by forensic examination before the Courts of Law
- Impart and Exercise relevant training program to the Investigating Officers, Judicial Officers, Medical Officers and other concerned state/central agencies
- Development of state-of-the-art methodologies to cater the need of analyzing challenging problems in the modern days forensic fields.

Biometrics in personal identification

Introduction

Biometrics is one of the most fascinating ways to solve the crime. It is an automated way to establish the identity of a person on the basis of his or her physical
finger print, face, hand/finger geometry, iris, retina, ear, etc.) and behavioral characteristics (signature, voice, gait, odor, etc.). Biometric technology makes a contribution to crime detection by associating the traces to the persons stored in the database, ranking the identity of persons and selecting subdivision of persons from which the trace may originate.

A biometric system is a pattern recognition device that acquires physical or behavioral data from an individual, extracts a salient feature set from the data, compares this feature set against the features set stored in the database and provides the result of the comparison. Therefore, a biometric system is composed of four modules:

Sensor module: This component acquires the raw biometric data of an individual by scanning and reading. For example, In-case of fingerprint recognition, an optical fingerprint sensor may be used to image the ridge pattern of the fingertip. **H**e quality of raw data is influenced by the scanning or camera device that is used.

Quality assessment and feature extraction module: For further processing, the quality of the acquired raw data is first assessed. The raw data is subjected to signal enhancement algorithm to improve its quality. This data is then processed and a set of salient features extracted to represent the underlying trait. This feature set is stored in the database and is referred as template. For example, the position and orientation of minutia in a fingerprint image is extracted by the feature extraction module in finger print biometric system.

Matching and decision-making module: In this module, the extracted templates are then matched against the stored templates and a matching score is given. On the basis of the matching score, the identity of a person is validated or ranked.

System database module: His module acts as storage of biometric system. During the enrolment process, the template extracted from raw biometric data is stored in the database along with some biographic information (such as name, address, etc.) of the user.

Concepts of biometric authentication

The selection of each biometric trait depends on the variety of issues besides its matching criteria. The seven factors that determine the suitability of a physical or behavioural trait to be used in biometric application.

1. Universality: Every individual who is using the biometric application must possess the trait.

2. Uniqueness: The trait must show sufficient difference across individuals comprising the population.

3. Permanence: The given biometric trait should not change significantly over a period of time.

4. Measurability: The trait should be easy to get and digitize and should not cause inconvenience to the individual. It should also be amenable to process further in order to extract features from the acquired data.

5. Performance: The recognition accuracy and the resources acquired to achieve that accuracy must meet the constraints imposed by the individual.

6. Acceptability: Individuals that will access the biometric device should be willing to present their biometric traits to the system.

7. Circumvention: It refers to the ease with which the trait of a person can be imitated or copied by using artefacts (e.g. fake fingers in case of physical and mimicry in case of behavioural traits). The biometric system should be immune to the circumvention.

Role in person identification

In the forensic context, a test sample obtained from a crime scene is referred as crime scene sample, traces material and questioned item whereas the reference sample that is compared against the crime scene sample is named controlled material or known item. Some of the trace samples (biological traces, finger marks, earmarks, bite marks and lip marks) are collected physically while others are acquired digitally (face, voice, body measurements and gait).

The particular biometric trait needs to be unique, distinctive and robust to the forensic conditions. Therefore, finger-marks and biological traces are searched in priority on a crime scene. The performance of a biometric system is largely influence by the quality of input sample conditioned by the acquisition and environmental conditions of a crime scene.

Accurate and reliable identification is an important issue in crime detection. The biometric recognition is emerging as a sound scientific justifiable tool in investigative procedure. It holds the potential to solve the criminal activities. The augmentation of wide varieties of criminal activities and advances in biometric technology mean that biometrics will have a more marked impact in crime detection in coming future.

Face Recognition

Another Biometric Technology includes the Face Recognition a technique that is capable of identifying or verifying a subject through an image. This method employs measures of face and head to identify the identity of a person through the facial biometric pattern. The first facial recognition was carried out in 1960 based on the features of eyes, nose, etc. The 21 subjective markers e.g. hair colour, lip thickness were employed by Goldstein and Harmon in 1970 to automate the recognition. The standard linear algebra techniques were applied in

1988 by Kirby and Sirovich for face recognition. The reasons that Face Biometric is beneficial than the Biometrics are:

- No physical interaction on behalf of the user is required.
- Accurate with high enrolment and verification rates.
- No experts as such needed for interpretation of the results.
- Only biometric that allows passive identification in a one-to-many environments.

Facial Recognition System

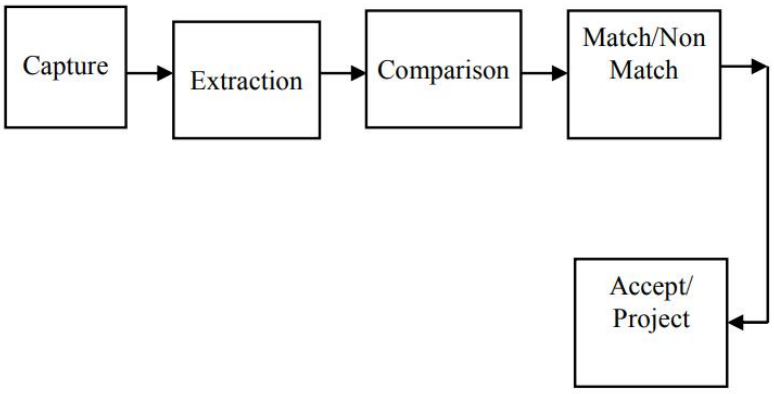
Two types of comparisons are done

Verification - It is comparison of the given individuals who are similar by the system and then result is yes or no decision.

Identification - The given individual is compared to all the entities present in the database thus deciphering a rank list of matches.

There are four stages in Identification technologies i.e.

i) *Capture:* During enrolment or verification or identification process, the system captures a physical or behavioural sample
ii) *Extraction:* The creation of template by extracting unique data.
iii) *Comparison:* Compare the template with a new sample.
iv) *Match/non-match:* Matching the features extracted from new sample and decide whether matched or not.



Stages of Facial recognition

Working

Everyone has distinguishable points on the face i.e. valleys and peaks which are defined as nodal points in software. There are 80 nodal points on human face that are measured by software i.e.

- distance between the eyes
- width of the nose
- depth of the eye socket
- cheekbones
- jaw line
- chin, etc.

For the general representation of face 70 nodal points are used to construct the face bunch graph and an image template is matched with this face graph in order to get the same points. The measured nodal points are given numerical code and strings of numbers represent a face in the database called face print.

Iris Geometry

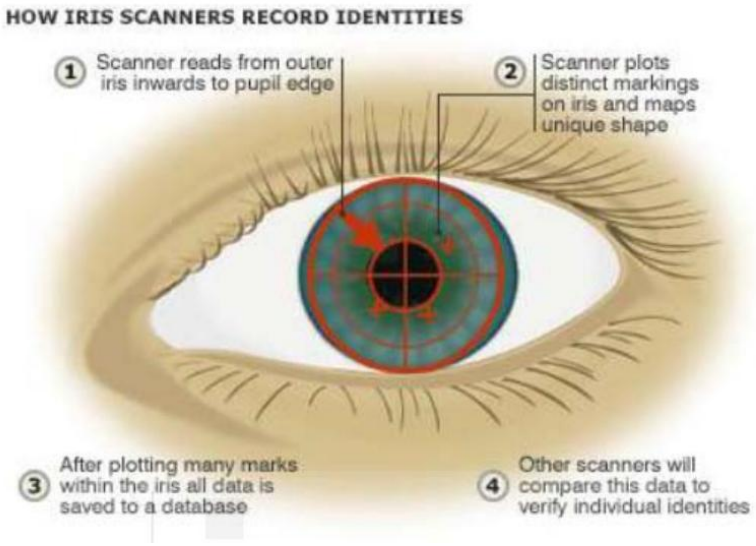
The uniqueness of the folds of muscles results into biometric authentication to confirm the identity with incredible accuracy. Other essentials you have to note is liveness detection like blink for scan enhance its accuracy and security. The technology has advantage of its applicability from a distance also. Note that the iris pattern is not subject to alteration with age, eye redness or alcohol consumption. Iris recognition can be used as Biometric because iris is the coloured portion of the eye surrounded by the pupil and its pattern is the result of meshwork of muscle ligament while colour and contrast are due to pigmentation.

Iris Recogntion

The recognition of Iris is carried out on the basis of the following traits:

- Iris scanning measures the iris pattern in the coloured part of the eye.
- Analysis of the iris of the eye, which is the coloured ring of tissue that surrounds the pupil of the eye.
- Based on visible features.

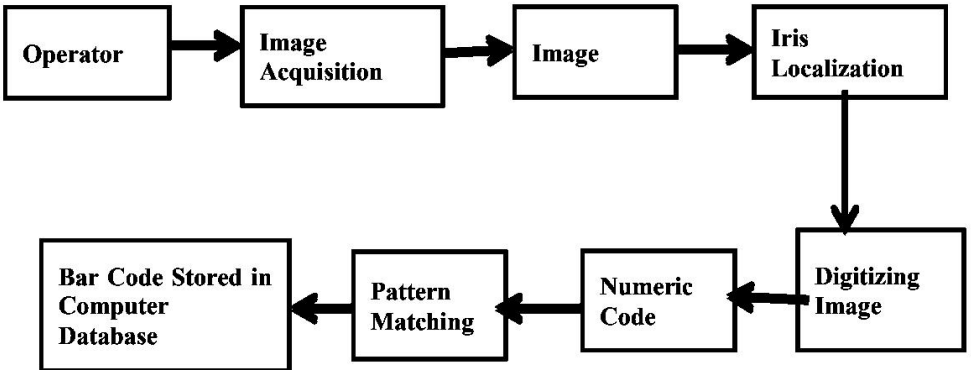
- Widely regarded as the most safe, accurate biometrics technology
- High speed, high accuracy.



Biometric Iris recognition is based upon the working of camera with the mathematical analysis of the random patterns. Imagine Iris as your phone camera where camera and light combine to take a photo. In the same way iris camera carries out a person's identity by combining computer vision, pattern recognition, statistical inference and optics.

This is how it happens:

- A person stands in front of the iris identification system with a distance of one or three feet so that the wide-angle camera calculates the position of their eye.
- The function of the second camera is to zoom on the eye and takes a black and white image.
- The focus of the iris overlays a circular grid on the image of the iris thus identifying the light and dark areas, like an "eye print".
- Fake eye prevention or fool system is done by the devices which vary the light shine into the eye and watch for pupil dilation.



Retinal Geometry

Dr. Carleton Simon and Dr Isodore Goldstein in 1930-1935 conducted first scientific study to describe how every individual possesses a unique Retinal structure. Because of the differing distribution of blood vessels, they suggested the use of the Retina to confirm the identity of an individual. Dr. Paul Tower (1950) reported that even identical twins have a very distinct and unique set of Retinas and has DNA Strand leading to very stable Biometric modality and that hardly changes.

Human retina is a thin tissue composed of neural cells located in the posterior portion of the eye. Retina is a complex structure with a network of blood capillaries and vessels resulting into the unique feature for every person as identical twins do not have same features. Retinal patterns are altered due to medical morbidities otherwise it is same from birth to death. The unique Retinal patterns of the individual is used as biometric identifier and the blood vessels within the retina absorb light more readily than the surrounding tissue which forms the typical smallest template for the Biometric Technology. The principle for the Retinal scan is carried out by casting an unperceived beam of low-energy infrared light (IR) into a person's eye and beam traces a standardised path on the retina as retinal blood vessels are more absorbent than the rest of the parts of the eye, then this path is converted to computer mode.

In comparison to Iris recognition where images are collected from a far distance but Retinal recognition requires end user must be in close proximity to retinal scanning device. The process has following steps:

1) *Image/Signal Acquisition and Processing:* The individual eye resides on tiny receptacle to receive infrared light beam for the illumination of retina at 3600. The duration of the process is few minutes with precaution that the individual must remain still till one rotation is

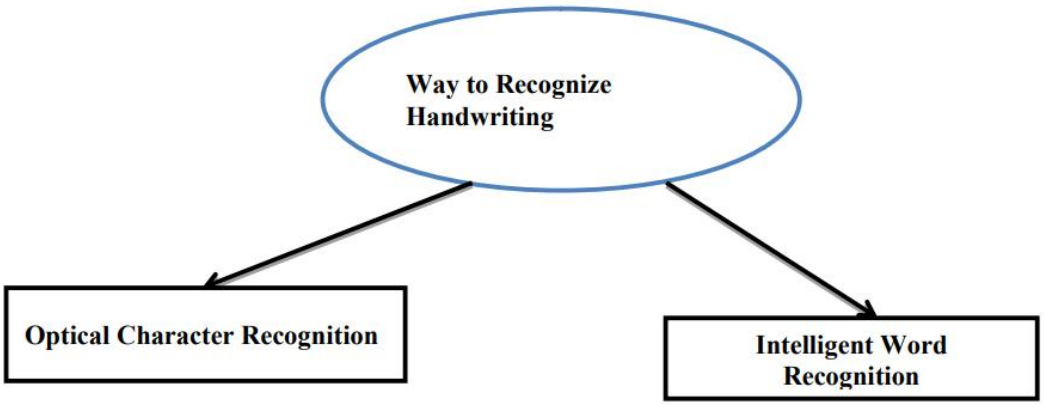
completed. Five images are taken to create the composite image from which the unique features are extracted.

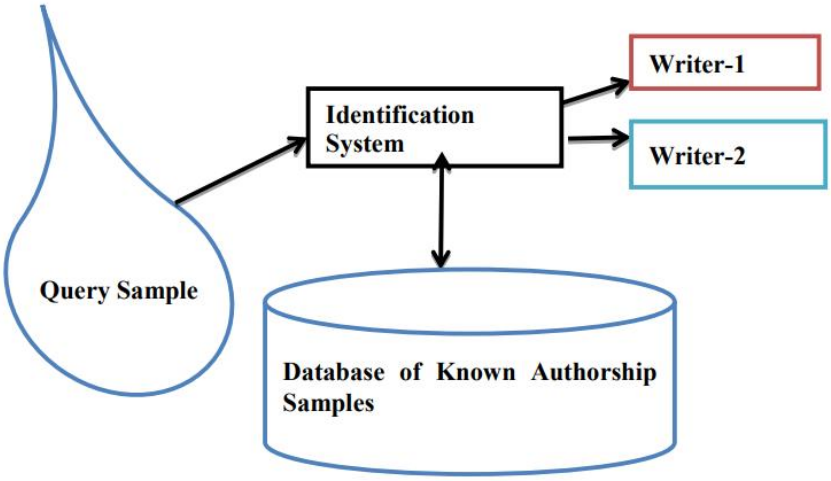
2) *Enrolments and Verification Template Creation*: Retinal patterns are not genetically controlled thus 400 unique data points are captured from the Retina, unlike the fingerprint, where only 30-40 unique data points are captured to create the Enrollment Template with a size of 96 bytes. The small size makes it possible to store number of templates in a single database and same process leads to creation of Verification Template. Due to the close proximity of numbers of factors influence the quality of Enrollment and Verification Templates as mentioned below:

- Individual cooperation is lacking
- The individual's eye and the lens distance are more.
- The size of the individual's pupil:

Hand Geometry

Handwriting detection is a technique operated by Computers with the processes to receive and interpret intelligible handwritten input from sources e.g. paper documents, touch screen, photo graphs etc.





Working

Two Types Recognition

- Online Recognition
- Offline Recognition

Online Recogntion

On-line handwriting recognition is based on the automatic conversion of text which is written on a special digitiser and the sensor with it picks up the pen up and down movements known as digital ink, thus converted to letter codes. The elements of the online recognition are:

- User can write with stylus or pen.
- A touch sensitive surface integrated with, or adjacent to, an output display.
- The interpretation of the movements of the stylus across the writing surface or translation of stokes into digital text is done by software.

Off-Line Recognition

Off-line handwriting recognition consists of the automatic conversion of text in an image into letter codes by the computers for text-processing applications regarded as static representation of handwriting. The different styles of writing make offline process difficult.

Recognition strategies are based upon the type of data and writing and first step is Character Recognition which is based on following criteria:

Pre-processing Methods on the data and includes the use of global transforms (correlation, Fourier descriptors, etc.), local comparison (local density, intersections with straight lines, variable masks, etc.); geometrical or topological characteristics The type of the decision algorithm relies on various statistical methods, neural networks, and structural matching Two strategies are employed for the word recognition:

1) The Holistic Approach - recognition is globally performed on the whole representation of words without segmentation thus an advantage of the method.

2) The Analytical Approach - deals with several levels of representation i.e. words are understood as sequences of smaller size units and easily related to characters in order to make recognition independent.

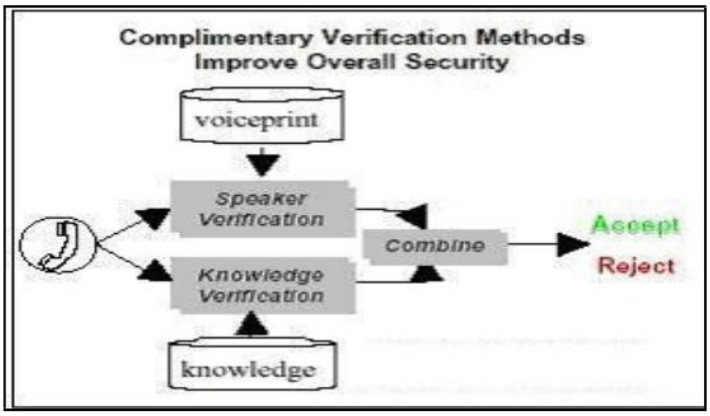
Handwriting Recognition objective is to design systems for recognition of handwriting of natural language. Methods and recognition rates depend on the level of constraints on handwriting and characterised by the:

- types of handwriting
- number of scriptors
- size of the vocabulary
- spatial layout

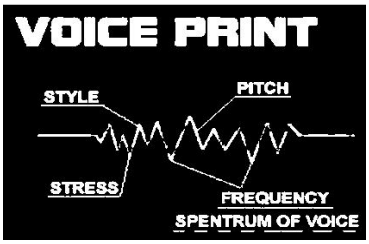
Speaker Recognition

Voice recognition is a crucial Biometric identifier as it recognises the speaker as well as the speech. The measurable, physical characteristic or personal behavioural traits are applied to verify and authenticate an individual. The two components, Speech recognition and Speaker verification are different as former identifies what you are saying and the later verifies who you are.

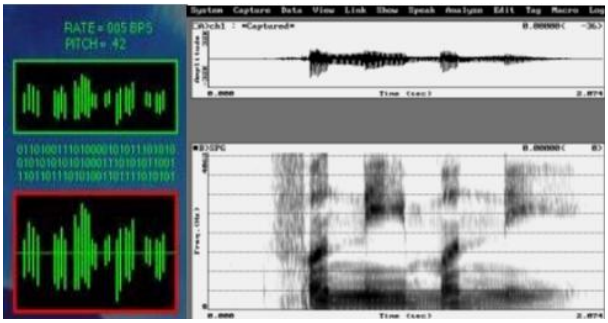
Biometric System is the combination of speech recognition and speaker verification systems



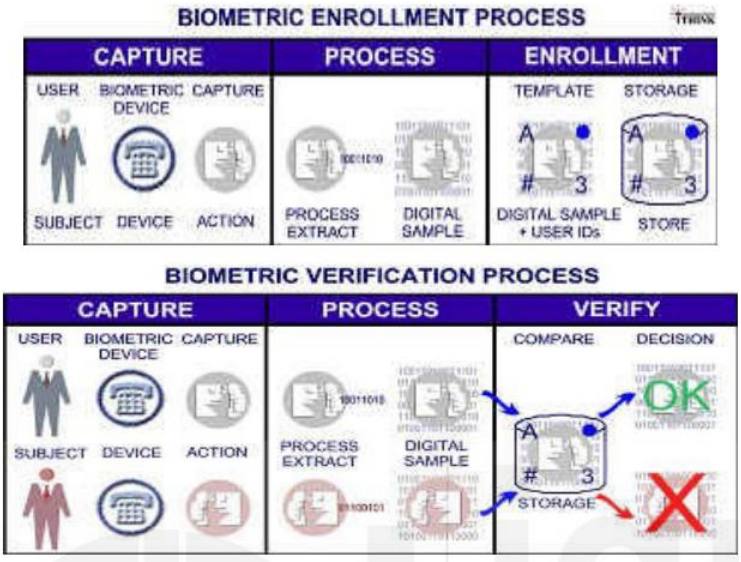
Voices have unique features like frequency and intensity which is manipulated in the system to digitising a person's speech to produce a “voice print”



In the second step these smaller segments are “captured” in a digital form and plotted on a spectrum.



Third step is the Verification Process stored in a table of numbers, where the presence of each dominant frequency in each segment is expressed as a binary number 0 and 1.



Signature verification

Handwritten signatures are widely used in everyday life. It serves as the means of persons identification and intent. It is distinctive and unique for each person. Although it is unique, it can be forged. For example, banks use signatures to verify cheques; art museums use signatures to identify the respective owners, and in many such scenarios, handwritten signatures are used. Thus, authenticating signatures is extremely important. A signature verification forensics test is a biometric process used to verify writing features like writing movements, strokes, pressures & the type of writing instruments (pen or pencil) and substrate (ink) used in the respective documents.

Signature verification in biometrics plays a significant role in forensic science, providing a reliable method for identifying individuals based on the unique characteristics of their handwritten signatures. In forensic investigations, signature verification is used to authenticate documents, detect forgery, and establish the identity of an individual involved in criminal activities. The process of biometric signature verification relies on both static and dynamic features. Static features include the physical appearance of the signature, such as the shape, size, slant, and pressure applied. These features are analyzed through visual examination or using automated image processing techniques. Dynamic features, on the other hand, capture the behavioral aspects of the signing process, including the speed, stroke order, pen pressure variations, and rhythm of the signature. These dynamic parameters are recorded through devices like digitizing tablets or smart pens, which provide detailed temporal data that cannot easily be replicated by a forger. Forensic examiners often combine both static and

dynamic analyses to ensure a robust comparison between questioned and reference signatures. In many cases, sophisticated algorithms based on machine learning are employed to enhance accuracy, helping distinguish between genuine signatures and forgeries. These systems can be trained to recognize subtle differences in signing patterns, making it difficult for an imposter to duplicate someone else's signature successfully. In addition to verifying signatures on legal documents, biometrics-based signature verification is crucial in cases of fraud, identity theft, and other criminal acts where handwritten signatures are used. The combination of traditional forensic examination techniques and modern biometric technologies has significantly improved the reliability of signature verification in legal and investigative contexts, providing forensic experts with powerful tools to uphold the integrity of evidence in courts of law.

Unit – 2

Fingerprinting and Forensic Serology

Fingerprinting

Fingerprints are the distinctive ridges appearing as corrugated lines on the tips of fingers and thumbs. The corrugation results due to rising of a portion of the upper layer of fingertip skin slightly above the normal level. Since the upper layer of skin is called epidermis, the finger ridges are also referred to as epidermal ridges. The depression between two ridges is called a furrow or a valley. The ridges and valleys form a complex, curved pattern on the fingertips.

The pattern on each finger of a person is so unique that it is not repeated on another finger of the same person or on the fingers of any other person. This makes fingerprints the most infallible means of identification.

General principles of Finger printing

The science of identification by screening and comparing fingerprints is called dactyloscopy. This science is based on the following three principles.

1. No two persons and no two fingers of the same person have identical ridge design on the fingertips. Fingerprints are absolutely unique – more unique than the DNA, the genetic material in the human cells. Although identical twins have same DNA sequencing, they do not have identical fingerprints.
2. The fingerprints remain unchanged throughout life. The ridge pattern begins to take shape during fetal stage and does not alter during a person's lifespan. It is only after death that decomposition sets in and the finger ridges are destroyed. Figures given below are the fingerprints of a child, recorded at the age of 2 and 15 years respectively. The prints do not show any variation.

Fingerprints of a child recorded with a time lag of about 13 years show no variations

In the growing age, the fingerprint pattern expands; as a result of aging, it may shrink. However, the basic design remains the same.

3. Fingerprints can be classified for record keeping. When a person commits a crime and is arrested, he is fingerprinted by the police. The fingerprint record is then passed on to the nearest fingerprint bureau. There are about twenty-five state level fingerprint bureaus in India. Their functioning is coordinated by Central Fingerprint Bureau, Ministry of Home Affairs. Each bureau maintains a catalogue of fingerprints.

Fingerprint detection

Fingerprint examiners use the ACE-V (analysis, comparison, evaluation and verification) method to reach a determination on each print.

Analysis involves assessing a print to determine if it can be used for a comparison. If the print is not suitable for comparison because of inadequate quality or quantity of features, the examination ends and the print is reported as not suitable. If the print is suitable, the analysis indicates the features to be used in the comparison and their tolerances (the amount of variation that will be accepted). The analysis may also uncover physical features such as recurves, deltas, creases and scars that help indicate where to begin the comparison.

Comparisons are performed by an analyst who views the known and suspect prints side-by-side. The analyst compares minutiae characteristics and locations to determine if they match. Known prints are often collected from persons of interest, victims, others present at the scene or through a search of one or more fingerprint databases such as the FBI's Integrated Automated Fingerprint Identification System (IAFIS). IAFIS is the largest fingerprint database in the world and, as of June 2012, held more than 72 million print records from criminals, military personnel, government employees and other civilian employees.

Evaluation is where the examiner ultimately decides if the prints are from the same source (identification or individualization), different sources (exclusion) or is inconclusive. Inconclusive results may be due to poor quality samples, lack of comparable areas, or insufficient number of corresponding or dissimilar features to be certain.

Verification is when another examiner independently analyzes, compares and evaluates the prints to either support or refute the conclusions of the original examiner. The examiner may also verify the suitability of determinations made in the analysis phase.

Powder tests – Dry powder method

Fingerprint powder is used on non-porous items of evidence to develop latent impressions, making them visible to the naked eye. This technique will enable the Forensic Scientist to lift, photograph or electronically capture the latent print for preservation and comparison purposes. Various processing techniques may be used after an item has been processed with powders. It is generally recommended that cyanoacrylate ester technique be used prior to powdering any item of evidence.

The powder technique for detecting latent fingerprints involves the application of a finely divided formulation to the fingermark impression, generally with a glass-fibre or a camel hair brush. The power gets mechanically adhered to the sweat residue defining the ridge pattern. The furrows which are devoid of the fingerprint residue do not adhere the power onto them. The final outcome is that the power formulation sticks to the ridges, but is easily blown off the furrows. Since the powder is normally colored, the ridge pattern becomes visible and the latent print is said to have developed.

Mechanism

The application of finely divided material and the sub-sequent removal of the excess power by brushing, blowing or tapping has been the universal method of intensifying fingerprints on non-absorbent surfaces since the early days of fingerprint technology. The technique relies on the mechanical adherence of fingerprint powder to the moisture and oily components of the skin ridge deposit.

The adhesion of powder formulation to fingerprint residue is governed by the pressure deficit mechanism. If a powder particle is wetted only on its lower side by the sweat deposition then owing to the curvature of meniscus there will be a pressure deficit inside the droplet, causing the particulate to adhere. The electrostatic attraction between the sweat residue and the powder particles, resulting due to frictional charges, also play a role in adhesion, albeit a minor one.

The effectiveness with which the powder adheres to the ridges depends on the size and shape of the particles that compose the formulation. Small, fine particles adhere more easily than large, coarse ones.

Therefore, most formulations are composed either of very fine, rounded particles (about 1 μm in diameter) or of fine flake particles (about 10 μm in diameter). Of late nanoparticle size powder compositions have proved to be very effective in lifting fingerprints.

As the age of the fingerprint residue increases the moisture and only components tend to evaporate, leaving the deposition more viscous. The same phenomenon is observed in warm climates. Thus, aged prints in tropical climates are relatively difficult to develop by power technique. The drying rate, however, is not dependent on the relative humidity indicating that the sweat residue has low water content near the surface.

Equipment and Materials

- Commercially prepared fingerprint powder: black, specialized powders and magnetic powder
- Brushes: camel hair (assorted sizes), Zephyr (fiberglass bristles), Feather, and Magna wands (used with magnetic powders)
- Fingerprint lifting tape and tape dispenser: clear, frosted, and polyethylene

Procedure

- Choose the powder that provides the most contrast. Powder shall provide the most contrast when compared to the background surface. Black, white, silver/gray, bi-chromatic, fluorescent, and magnetic powders are all available for use. Additionally, depending upon the material of the item (metal, plastic, etc.), the magnetic properties of the powder shall be considered. Magnetic powder may be used on nonmetal items.
- Dip brush into the powder containing vessel. Ensure that the tips of the bristles make contact with the powder surface.
- For non-magnetic powders, tap away excess powder from brush.
- Lightly brush the powder over the surface of the item of evidence using only the tips of the brush. Carefully brush the entire surface of the item until ridge detail becomes visible. Some latent impressions are light in color and will not appear dark after

powder is applied. Low levels of moisture in the latent print may account for this. An effective way to develop these types of impressions is to introduce moisture to the latent print. This may be accomplished by breathing moist air onto the area containing the latent print. Allow the moisture to dry and reapply the powder (it is not necessary to allow the moisture to dry when using magnetic powders).

- Once the latent impression has become visible, gently brush away any excess powder adhering to the impression. Impression shall then be preserved using photography, lifting techniques or electronic preservation methods.
- *Photography:* It is important to photograph any developed impression prior to lifting attempts. This will assist the Forensic Scientist at a later time if the impression is damaged or destroyed by further processing. A variety of camera equipment is available to record the impressions (see Technical Procedure for Nikon Digital Camera). Use a scale in all photographs.
- *Lifting:* The impression may be lifted by applying the adhesive side of a commercially available lifting tape to the surface containing the latent print. The tape used must be large enough that one piece covers the entire area to be lifted. A folded over flap of tape shall be used for a hand-hold.
- Tape shall be removed from the roll in one motion in order to prevent streaks.
- Apply tape evenly in order to prevent wrinkles and/or air bubbles. Firmly hold the folded-over end of the tape, slowly press the free end onto the surface to be lifted and smooth the length of tape over the surface.
- Tape shall then be removed in a smooth, continuous motion.
- Place the tape on a sheet of white paper (or lift card which is of contrasting color) in the same manner as the tape was applied to the surface. When several prints are developed (side by side or grouped closely together), lift all on one piece of tape. Wider tapes shall be used for multiple prints (or place two or more strips of tape over the impression and lift together).
- Label each lift with the appropriate case numbers, item number, date, Forensic Scientist initials, location the lift(s) originated and any other cross reference information necessary.
- *Electronically preserved impressions*: Latent impressions may also be electronically recorded with a computerized system such as the Image Processing System (See Section Image Processing Procedure). This process is to be used with faint or difficult

impressions on certain surfaces. This practice often will allow the Forensic Scientist to record impressions that may have been unrecoverable prior to the implementation of this technology.

Detection using Cellophane tape

This procedure can be used to develop impressions on the adhesive side of duct tape, masking tape, clear plastic tape, plastic surgical tape, reinforced packing tape, packing labels and black electrical tape.

Procedure

Plastic-Backed Adhesive Tape

- Examiners shall produce a self-made test print to be processed concurrently with items of evidence. (See section technical procedure for Ensuring Quality Control).
- Place tape, adhesive side up, in a plastic or glass container that is large enough to accommodate the evidence.
- Pour the tape into the container so that the tape is completely covered by the solution. Allow solution to remain on tape for 10-15 seconds.
- Remove the tape from the solution and rinse with tap water to remove the excess chemicals and allow drying completely prior to proceeding. (Distilled water may be used at a crime scene, but it is not a requirement). Examine with an alternate light source.
- Alternate Process One - Use a disposable foam applicator or a camelhair brush to gently brush TapeGlo onto the adhesive surface. Rinse and examine with an alternate light source.
- Alternate Process Two - Place TapeGlo liquid in a spray bottle and spray TapeGlo onto the adhesive surface. Be sure that the adhesive surface is completely covered with a thin layer of solution. Rinse and examine with an alternate light source.

Chemical tests

Chemical tests play a significant role in the identification of latent fingerprints in forensic investigations. These techniques rely on the interaction between chemicals and the

components of fingerprints, such as sweat, oils, and amino acids, to make the prints visible. One common method is the use of ninhydrin, which reacts with amino acids in sweat to produce a purple or pink color, making the print visible on porous surfaces like paper. Another is silver nitrate, which reacts with the salt in sweat to form silver chloride, leaving a visible print when exposed to light. Cyanoacrylate fuming, or "superglue fuming," is another widely used method, where fumes adhere to the oils in the print and create a white polymer coating. These chemical tests are crucial in lifting latent prints from various surfaces where conventional methods, like dusting powders, may be less effective.

Silver nitrate test

Silver nitrate technique is one of the oldest methods of detecting latent fingerprints on porous items like paper, cardboard and wood. The method is based on the reaction of silver ions with the chloride content of sweat residue. The reaction product, silver chloride, is relatively unstable and, when exposed to ultraviolet radiation or sunlight, decomposes into finely divided silver, visualizing the ridges as a dark hue, usually black or brown.

Mechanism

Detection of the latent fingerprints by silver nitrate is based on the premise that the said reagent undergoes chemical interaction with the chloride content of sweat residue. This reaction produces a precipitate of light sensitive silver chloride. When white colored, insoluble silver chloride is exposed to either sunlight or ultraviolet radiation, it gets reduced to metallic silver.

$$AgNO_3 + Cl^- \rightarrow AgCl + NO_3^-$$

$$2AgCl \xrightarrow{h\nu} 2Ag + Cl_2$$

The finely divided black or brown colored metallic silver adheres to the ridges and visualizes the fingerprint.

Methodology

The silver nitrate reagent develops fingerprints on porous surfaces, such as papers of different varieties, cardboard and raw (unpolished) wood. The reagent is dissolved in distilled water or

in a mixture of distilled water and methanol. Tap water is not used since it invariably contains chloride ions which transform silver nitrate to silver chloride. The concentration of silver nitrate in test solution may vary from 1-10%. However, a 3% solution (w/v) is considered to be the best choice. The latent fingerprint-bearing item is treated with the test solution either by dipping or by spraying.

After the fingerprints develop, the article should once again be washed with water so as remove excess silver nitrate. Otherwise, the silver ions adhering to the surface are slowly transformed to metallic silver. As a result, the entire surface gets stained and consequently the contrast between the developed print and the background is substantially reduced.

Nevertheless, the staining of the background cannot be completely avoided. Since the method is applicable to porous items, some silver ions inevitably seep into the capillaries of the substrate and eventually impart a dark color to the background. To avoid this problem, it is recommended that as part of post-development, the item should be first washed with water and then area outside the periphery of fingerprint be treated with a stopping solution. The latter delays, the darkening of the background. The most commonly used stopping solution is that of mercuric nitrate (3% w/v in distilled water). As the silver ions absorbed into the body of substrate are transformed into metallic silver, the mercuric nitrate reverses the reaction. That is, the metallic silver, which would have stained the background, is re-converted to soluble silver nitrate, which is subsequently drained off.

$$2\,Ag + Hg(NO_3)_2 \rightarrow 2\,AgNO_3 + Hg$$

Assessment

As with other chemical methods of fingerprint detection, the silver nitrate technique has both advantages and disadvantages. The major advantage of this method is that the chloride ions in latent fingerprints are non-volatile and therefore fingerprints which are almost one week old may be developed by silver nitrate reagent. However, after 7-10 days, the chloride ions of sweat residue tend to diffuse into the body of the substrate and the prints developed by silver nitrate are not of optimum quality.

The ninhydrin and iodine fuming methods of fingerprint detection do not interfere with the silver nitrate technique. Thus this method may be used as a final step to reveal fingerprints when the other conventional methods do not yield results. In fact, if the fingerprints

developed by ninhydrin on a particular surface are weak and faint, the item may be post-treated with silver nitrate to give a better contrast.

The main demerit of the silver nitrate method is that although the developed prints are of intense color, the background is stained and, over a period of time, the contrast is lowered. The staining may be delayed by either post-treating the background area with a stopping solution or by preserving the developed prints in a dark chamber. Silver nitrate is a costly and a toxic chemical. On coming in contact with skin, it produces a black mark which persists for several days.

Iodine fuming test

The iodine fuming technique for visualizing latent fingermarks has been used for almost a century. Iodine is a crystalline solid, which upon heating sublimes into violet vapors. The vapors are absorbed by the sweat deposition of the latent imprint thereby revealing the ridge pattern.

Initially, it was believed that iodine can be added chemically and reversibly, to double bonds of the unsaturated fatty acids present in the fingerprint residue. However, it was later suggested that the mechanism of the reaction involved physical absorption of iodine on the fatty acid content. The iodine-fatty acid interaction imparts a yellowish brown color to the ridge pattern. Iodine fuming is a simple procedure of developing latent fingerprints.

Methodology

Latent fingerprints may be developed by iodine fuming technique by one of the following experimental procedures:

a. Fuming Gun Method: An iodine-fuming gun is made of either glass or plastic. The butt-end of the gun contains a dehydrating agent, usually calcium chloride. There is also a mouthpiece for blowing air at this end.

Near the nozzle-end of the gun about 0.5 g iodine crystals are placed. Air is blown though the mouthpiece and the gun nozzle is moved above the surface impinged with the latent prints. The optimum distance between the gun and the surface is 2 cm. As soon as the latent

fingerprints begin to appear, the iodine fumes are concentrated over the imprinted area. The prints so developed are photographed immediately.

b. Fuming Cabinet Method: A china dish containing about 1g iodine crystals is placed in a fuming cabinet. The object bearing the latent prints is suspended from the roof of the cabinet. Iodine crystals are gently heated to about 50° C using a heating block. After a few minutes latent fingerprints begin to appear. The object is exposed to the fumes till maximum contrast has been achieved between the prints and the background. The developed fingerprints are immediately photographed.

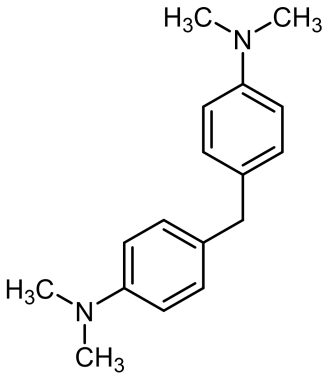
c) Powder Dusting Method: Iodine crystals are pulverized into a fine powder. The power is spread out over the surface bearing the latent fingerprints by a camel hair brush. The excess powder is blown off. This method does not require any equipment and is, therefore, both simple and cost-effective. However, it exposes the user to the toxic fumes of iodine. As a result, this method is now obsolete.

Post development of fingerprints

Fingerprints developed by iodine are not permanent in nature. These tend to fade out on standing. In presence of air the fading of prints is accelerated. For this reason the iodine developed prints have to be photographed immediately. Nevertheless, it is possible to fix the prints by using iodine in conjunction with other chemical reagents.

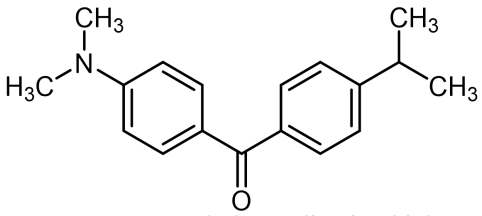
Iodine is known to react with starch giving a stable, deep blue complex. Thus, posttreatment of iodine-developed fingerprints with starch solution gives long-lasting blue impressions. The fading problem may also be avoided by pressing a silver foil onto the iodine-developed fingermarks. The interaction of iodine, absorbed on the fingerprint residue, with silver produces yellow colored silver iodide. The latter, on exposure to light decomposes into finely divided silver, revealing the ridge pattern as a stable, black deposition.

Iodine exposed fingerprints by may be fixed post-treatment with a solution of N,N,N’,N’-tetramethyl-4,4’-diaminodiphenylmethane in 1,2,2-tricholorotrifluoromethane.

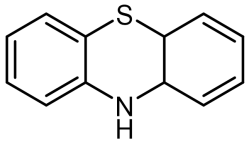


The color of the prints change from brown to green-blue. Fig. 6 shows an iodine developed fingerprint, half of which was treated with N,N,N’,N’-tetramethyl-4,4’-diaminodiphenylmethane reagent. The treated part is blue in color, while the untreated part is brown.

Nevertheless, the quality and clarity of post-treated fingerprints are not affected. Iodinedeveloped fingerprints on thermal paper may also be fixed by spraying with a hexane solution N,N,N’,N’-tetramethyl-4,4’-diaminothiobenzophenone or phenothiazine. With former, the color of developed prints changes to blue, while with latter, reddish impressions are obtained.

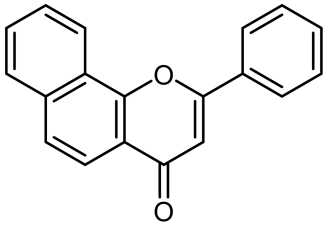


N,N,N',N'-tetramethyl-4,4'-diaminothiobenzophenone



Phenothiazine

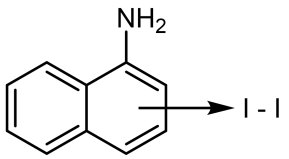
The most common reagent for fixing iodine-developed prints is 7,8-benzoflavone, the structure of which is shown in Figure. A mixture of 2 ml of 10% 7,8-benzoflavone solution in dichloromethane and 100 ml of 0.1% iodine solution in cyclohexane is used for this purpose.



7,8 Benzoflavone

Iodine in combination with 7,8-benzoflavone reagent may be used for developing old latent fingerprints on porous surface, such as bond paper, newsprints and facial tissue paper. The composition is non-destructive and the documents remain unaltered.

Sublimable or low boiling organic reagents may also be made to interact with and fix iodine absorbed on the latent fingerprints used in vapor state. For example, iodine interacts with **α**-naphthyl amine to form a red colored charge-transfer complex. Although free iodine does not possess a natural dipole moment, yet in concert with an aromatic system, it acquires an induced dipole. The combination of electron-rich aromatic compounds and dipolar iodine results in charge-transfer complexation. The charge transfer complex is non-volatile in nature and hence it does not evaporate from the frictional ridges. As a result, the developed prints do not fade out with passage of time.



Assessment

The iodine method for detecting latent fingerprints has the following advantages:

- The procedure is very simple to use and requires only a meager degree of skill and experience.
- With conventional chemical developing reagents, the latent fingerprints, on the surface in question, are first visually searched and then the relevant spray or powder formulation is applied. However, while using the iodine method, there is no need to first scan the surface. Iodine vapors on their own get preferentially absorbed on the fingerprint residue, revealing the ridges.
- A number of chemical fingerprint development methods, such as ninhydrin, cyanoacrylate and silver nitrate require instrumental detection techniques, such as laser and neutron activation analysis, and therefore cannot be used for on-thescene investigations. The object bearing the latent fingermarks has to be carried to the fingerprint laboratory if any of these methods is desired to be used. However, iodine

in conjunction with 7,8-benzoflavon, may be used for detection of fingerprints at the scene of crime.

- Iodine fuming is a sensitive method for developing latent fingerprints on porous surface, such as paper and paper products. The other reliable method for porous surface is the ninhydrin technique. However, good results are obtained by ninhydrin only after the developed fingerprints are treated with metal salts, following by laser examination. No such costly equipment is required with iodine.
- Iodine fuming develops both fresh and relatively old prints.
- Iodine fuming is one of the techniques, which can develop fingerprints on human skin. The area of skin suspected to be impinged with latent print is treated with iodine vapors. Once the impression appears, these are pressed on to a silver plate. Exposure of the plate to light reveals permanent fingermarks.
- If in a particular casework investigation, iodine method fails to detect fingerprints on crime scene evidence, other techniques like ninhydrin method may still be tried out.
- Iodine method suffers from the following shortcomings:-
- The iodine developed fingerprints fade out in a short time. Hence these have to be fixed using a suitable reagent. Yet, it is pertinent to state here that other fingerprint detecting techniques, such as powder dusting, ninhydrin spraying and cyanoacrylate fuming too give good results only after post-treatment operations.
- Iodine is toxic and poses occupational hazards to the user. Nevertheless, by observing some simple safety precautions, this problem may be eliminated.

Ninhydrin test

A latent fingerprint is formed when the sweat pores of the papillary ridges leave a deposit of perspiration on a surface with which the finger has been brought into contact. Human skin possesses three types of sweat glands: Ecrine, apocrine and sebaceous, the secretions of which contribute to fingerprint deposit.

Ecrine glands are widely distributed throughout the body and are particularly numerous on the palms of the hands and the soles of the feet. Besides the water content, eccrine sweat contains up to 1% of other substances of which about one-half are inorganic ions. The remaining half consists of organic derivatives like urea, sugars, lactic acid, fatty acids and amino acids/proteins.

The amino acid content of sweat residue may be fixed by treating the latent fingerprint with a solution of ninhydrin reagent. The amino acid-ninhydrin reaction produces a purple compound, called Ruhemann's purple, which becomes deposited along the ridges, making the latent prints visible.

Ninhydrin is particularly useful for developing fingerprints on porous and absorbent surfaces like paper, paper products, cardboard and wood. Whereas the inorganic ions and most of the organic ingredients of sweat deposit tend to interact with the cellulose content of paper or wood, the amino acids remain inert. Moreover, with passage of time the amino acids do not migrate within the capillaries of the substrate.

Ninhydrin as a fingerprint agent

Broadly, the procedure for developing latent fingermarks involves spraying the ninhydrin solution on the surface containing the impression, from a distance of about 6 inches. After the solvent evaporates, the solution is re-sprayed. The surface is then heated for a short time, without allowing it to come into contact with the heat source. If quick development is not required, the item may be allowed to dry at room temperature. Better results are obtained by natural drying.

During experimentation, there are several parameters that need to be adjusted for adequate and satisfactory development of fingerprints. There are chemical parameters, such as the concentration of ninhydrin, the nature of solvent and the acidity of the formulation, as well as physical parameters, such as the mode of application, heating temperatures and relative humidity.

(a) Chemical parameters:

Concentrations of ninhydrin varying from 0.2 to 1.5% have been found suitable for fingerprint development. Various types of solvents have been tried out for preparing ninhydrin solutions. The reagent dissolves in most organic solvents, such as methanol, ethanol, acetone, diethyl ether and naphtha. The choice of the solvent depends, in part, on whether the document is scripted or not. Most organic solvents tend to despoil the calligraphic script.

Freon-113 (also called fluorisol) proved to be the most remarkable solvent for dissolving ninhydrin. It does not interact with ink. It is absolutely non-reactive and therefore there is no chance of the surface becoming degraded. It is non-toxic and therefore poses no occupational hazard to the user. Above all, it is non-flammable and therefore quite safe to use in a laboratory.

As a result of this last characteristic, the Freon-based composition has come to be referred to as non-flammable ninhydrin or NFN. The NFN develops fingermarks on a wide range of paper items. It provides a minimal amount of background effects. Moreover, it is effective on surface-coated papers, such as currency notes, as well as the gummed side of postage stamps. It is, however, being feared that it may not be possible to continue with NFN in the years to come.

Freon-113 belongs to a class of compounds called chlorofluorocarbons. These compounds are being banned out under the terms of Montreal protocol on phasing out of ozone depleting substances. There is therefore, a need for a suitable replacement of Freon-113. Petroleum ether provides an alternative, as it too does not interact with ink. However, petroleum ether is highly inflammable and explosive in nature and is therefore considered hazardous. Supercritical carbon dioxide is being considered a possible substitute of Freon-113.

Post-treatment of Developed Fingerprints

Although the ninhydrin method is considered to be a standard procedure for developing latent fingerprints, it suffers from a number of demerits. The chemical reaction between ninhydrin and the amino acid content of sweat deposit is quite slow and therefore, the developing time is quite long.

Many surfaces interact with ninhydrin, giving a strong background coloration that masks the developed prints. Further, the concentration of amino acids in perspiration is quite low and hence the developed prints usually do not show a sharp contrast. Moreover, the method is not applicable to non-absorbent and dark surfaces. Lastly, on many items, the ninhydrin-developed prints tend to fade out within a very short span of time.

In order to avoid these problems, and to improve the working performance of ninhydrin reagent, the procedure for fingerprint development may be modified. The modifications involve the conversion of non-fluorescent Ruhemann's purple (VII) deposited along the

ridges, into a fluorescent derivative by post-treatment of the developed prints with a suitable metal salt. The fluorescent deposition shows a marked enhancement in clarity and sharpness when illuminated by a light source.

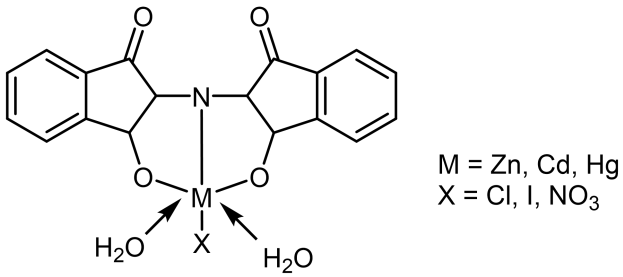
Post-treatment with metal salts in concert with arc lamps

Ruhemann's purple (RP), the reaction product of ninhydrin with amino acids, forms coordination compounds with many metals. The metal-RP complexes are generally red or orange in color. Some of these are fluorescent in nature. This reaction may be used to overcome the contrast problem of ninhydrin-developed prints, as well as to improve the stability of fingermarks.

The reaction of Ruhemann's purple with a Group 12 metal salt, like halides or nitrates of zinc(II), cadmium(II) or mercuric(II), in an ethanol-Freon-113 solvent mixture, produces a photo-luminescent complex [VIII; M = Zn (II), Cd (II) or Hg (II); X = halide or nitrate ion].

However, invariably, zinc chloride is the reagent of choice for post-treatment operations. After the solvents evaporate, the sample is dipped in liquid nitrogen so as to bring down the temperature to -200° C.

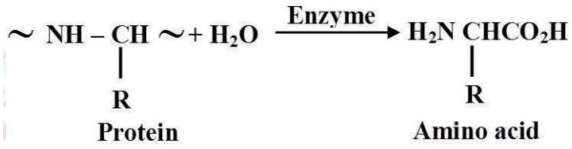
When examined under an arc lamp, intense fluorescence is produced. A xenon lamp is the most common source for illuminating prints developed by ninhydrin and post-treated by zinc chloride. A Watkin's indium lamp, a Kent's Quaser lamp or a Unilite lamp may also be used for illuminating the fingermarks.



This procedure offers several advantages. The post-treatment may be carried out only when it is deemed essential. If a sufficient degree of contrast is produced with ninhydrin alone, the subsequent steps may be avoided. No expensive equipment like a laser is required. Further, prints may be photographed using conventional fingerprint cameras.

Pretreatment of developed fingerprints

The amino acid content of sweat deposition is quite small and therefore ridge details are often not visualized clearly with ninhydrin, even after post-treatment with zinc chloride and laser examination. To improve the sharpness of the developed prints, pre-treatment with hydrolytic enzymes like trypsin may be tried. These enzymes hydrolyze the protein content of perspiration to amino acids, thereby increasing the chances for enhancement of developed fingerprints.



Initial attempts to pre-treat the fingerprints samples proved futile because the enzymes were dissolved in non-aqueous solvents in which they denatured. Subsequently, however, the dusting of powdered enzymes before the ninhydrin treatment was tried. This gave pronounced enhancement of latent fingermarks, especially those that were 2 weeks to 3 months old.

The methodology involves the following steps: The enzyme is applied by dusting over the fingermark and the sample is incubated for about 40 minutes at 35-40° C under a relative humidity of 80-90%. The article is then dried and excess enzyme is blown off.

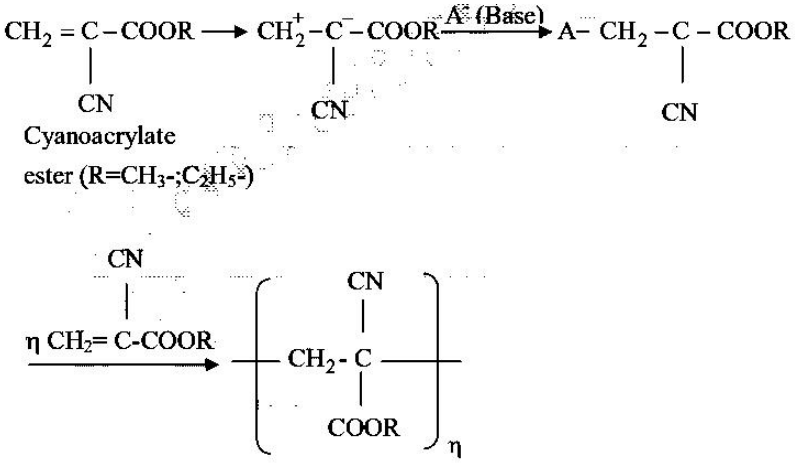
This is necessary since the enzymes too react with ninhydrin giving colored compounds. The dried, enzyme-free sample is treated with ninhydrin and re-incubated for about 18 hours. If ridge details are still not clear, further treatment with zinc chloride, followed by laser examination may be carried out.

Enzyme pre-treatment method cannot be applied to routine samples. It requires a long developing time. It is unsuitable for fieldwork. The technique may be tried out over articles bearing extremely weak fingerprints.

Superglue (Cyanoacrylate) test

The principle underlying the cyanoacrylate method (also called Super Glue technique) depends on the fact that when alkyl 2-cyanoacrylate reagent is allowed to vaporize, it

undergoes polymerization. The polymerized ester has a tendency to get adsorbed on the sweat residue, imparting a white color to the ridge pattern.

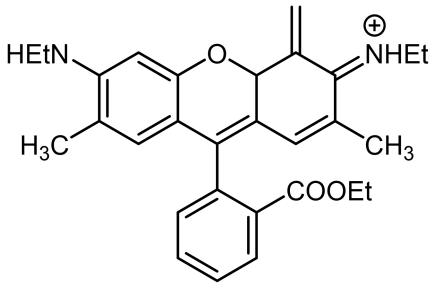


A china dish containing a few drops of cyanoacrylate and sodium hydroxide solution is placed in a fuming cupboard. The object bearing the latent prints is suspended from the roof of the cabinet. The item is exposed to cyanoacrylate vapors for about 2 hours until whitish colored fingerprint pattern develops.

Even though cyanoacrylate fuming is a convenient and reliable method for detecting fingerprints, the developed imprints are white in color and, therefore, lack contrast. The contrast may be enhanced by post-treating the cyanoacrylate-developed fingerprints with a solution of a luminescent dye. Such stains fluoresce upon exposure to laser light, revealing sharp fingerprints.

When dye solutions alone are used for print processing, the results are not satisfactory. The reason being that the dye solution tends to wash off the sweat residue. However, when stains are used in conjunction with cyanoacrylate fuming, this problem is avoided. The cyanoacrylate polymer stabilizes the latent prints, so that solution dye staining does not wash it away.

Crystal violet, (VI) improves the contrast of weak prints developed on polythene by cyanoacrylate method. The stain gets selectively adsorbed on the polymerized cyanoacrylate. Likewise, the rhodamine 6 G (IX) treated prints also get preferentially adhered to the polycyanoacrylate deposition and hence are suitable for laser examination. Moreover, the absorption spectrum of the stain matches with the blue-green illumination of the Ar-laser.



Ruthenium oxide test

Ruthenium tetroxide (RuO_4, also known as RTX) is an inorganic oxide that adheres to fingerprint residue. A handful of scientific articles report the use of RuO_4 fuming or direct application as a method to reveal latent prints. RuO_4 can be produced in situ by mixing aqueous $RuCl_3$ and ammonium cerium (IV) nitrate $(NH_4)_2Ce(NO_3)_6$. The resulting tetroxide is insoluble in water and is released. RuO_4 may also be applied in a halogenated hydrocarbon solvent. Evidently, RuO_4 is attracted to the fatty fingerprint residues forming a dark colored ruthenium dioxide in the presence of fatty fingerprint residues. The chemical mechanism for this transformation is unknown.

Forensic Serology

Forensic biology and serology is a branch of forensic science which deals with biological evidences and their examination. In the examination of crimes such as murder, robbery, rape etc. the examination of biological materials play an important role in connecting the criminal with the crime. Such biological specimens may be in the form of body fluids, stains or other material. These materials are:

1. Blood
2. Semen
3. Saliva
4. Urine
5. Faecal matter
6. Milk
7. Hair.

The most important evidences of body fluids are blood and semen from personal identification point of view.

Blood types

Blood is a complex viscous red fluid with a pH of about 7.4. It is mainly composed of two parts: Cells and plasma. The liquid part is called plasma and the solids are red cells (erythrocytes), white cells (leukocytes) and thrombocytes (platelets). The cells are also known as corpuscles. When the blood flows out of the body a part separates out as blood clots consisting of blood cells, discs and fibrin. Fibrin comes out of plasma and is responsible for clotting of blood. The liquid left is called serum. It is the plasma without fibrinogen which turns into fibrin.

a) Red cells: There are about five million red cells per micro litre of blood, each about ten micron in diameter. The red cells are continuously being produced and destroyed in the body. The rate of destruction is about ten billion cells per hour in a normal healthy adult.

b) Haemoglobin: The colouring substance of the red blood cells is haemoglobin. The substance is of great importance to the body functions as it is the carrier of oxygen, injected medicines, salts and food to various body tissues. It is useful in blood examinations.

c) White cells: The number of white cells is about four to eleven thousand cells per micro litre. They resist the attack of diseases.

d) Platelets: The number of platelets is about five times that of white cells. They facilitate blood clotting.

e) Serum: Plasma and serum are complex mixtures of proteins, minerals and organic compounds dissolved in water.

Characterisation of Blood stains

The identification and evaluation of blood is based in its composition and behaviour under various conditions.

1) *Cell structure:* Since blood is composed of red and white cells, platelets and plasma. The cells have definite shapes and sizes which in different species. For example, human blood cells have characteristic non-nucleated discs with a diameter of about 0.08mm. The identification of cell structure is possible when the blood is fresh and moist usually less than

1 day old. When the blood has dried, it is not always possible to completely regenerate the blood cells. The study of cells is made through microscopy.

2) *Enzymatic activity*: Blood contains heme group in haemoglobin. It behaves like peroxidase. It catalyses the liberation of oxygen from oxygen rich compounds like hydrogen peroxide or sodium perborate. The oxygen is used to carry out certain color reactions. The common color reactions for blood detection are benzidine, phenolphthalein, leucomalachite green and luminol reactions. Other enzymes have been utilized in electrophoretic techniques. When they are subjected to an electric field due to some polarity, they move towards opposite polarity. The rate of movement varies with their structure, weight and electric charge. The pattern of separation is characteristic of blood.

3) Spectrophotometry Haemoglobin on treatment with acids, alkalis, reducing agents or oxidizing agents gives a variety of products which have characteristics absorption spectra. They help to identify the blood. The absorption bands are seen in length which are characteristic of blood.

4) Visual examination It is useful to determine whether the given stain is of blood or not. Ordinarily, there is no difficulty in fresh but old stains may not be identifiable. The examination permits:

- Determination of number and size of stains.
- Rough estimate of amount of blood shed.
- The direction from which the blood has fallen is found from the tip of the elongated stain. The tip of the elongated points out the direction.
- The shape of the stain indicates the height from which the blood has fallen.
- Bloodstains from the height of upto 50 centimetres are round sharp edges.
- Bloodstains from a height of 50 to 150 centimetres have small spike like projections along the edges.
- Bloodstains fallen from a height over 150 centimetres have corrugated edges.
- From the positions of the stains, the movement of the victim and the culprit can be determined. If the culprit ran away bleeding, he can be tracked through blood stains.
- The degree of fluidity, dryness and changes in color permits rough estimation of age of the stains.

- Gross foreign matter like hair, flesh, bones in the blood may identify the site of injury.

5) *UV and IR Examination*: Ultraviolet or infrared rays reveal washed or invisible bloodstains from clothes, furniture, earth, doors etc. They also reveal stains on coloured garments or on painted surfaces. They help to reveal minute blood traces.

6) *Microscopy*: Microscopic examination of bloodstains is important in many situations:

- Species of origin of fresh bloodstains can be established through microscopic studies and micro measurements sometimes.
- The part of the body from which the blood has come can found out from the nature of extraneous matter. Thus, blood from nose, mouth, vagina or anus can be identified.
- Diseases like leukaemia or syphilis can be detected microscopically.
- Menstrual blood can be identified.
- Blood from an infected site can be identified through the presence of puss.

7) *Spectroscopy:* Spectroscopic examination of blood is very useful as well as convenient. The test is usually carried out microscopically. The blood haemoglobin is changed in two or three forms on the slide itself and characteristic absorption are observed. Usually alkali hematin and cyanhaemochromogen are studied for their characteristic spectra.

8) *Chromatography:* Ascending paper chromatography using acetic acid, methanol and water solvent system has been employed to study the Rf values.

9) *Electrophoresis:* It is used for separation of various enzyme systems. It is being adopted to study the body proteins. This technique is becoming important to distinguish between blood samples.

10) *Colour tests:* These tests are the first series of tests employed after visual study of stains. If a stain gives positive color reactions in any of the two color tests, the stain is possibly a bloodstain. If it fails to give color reactions in all probability it is not a bloodstain or the same cannot be established.

Benzidine reaction: Benzidine (0.1g) and dry sodium perborate (0.1g) are dissolved in glacial acetic acid (10ml) and sprayed over the stain. A strong blue color indicates blood.

Leucomalachite Green reaction: The reagent is prepared by dissolving leucomalachite green (0.1g), sodium perborate (0.3g) in 65% glacial acetic acid. The reagent is applied to the stain. Intense green color indicates blood.

Phenolphthalein reaction: Phenolphthalein (1g) is reduced and dissolved in acetic acid (100ml). Sodium perborate (1.4g) is dissolved in the solution and applied to the blood stain. Pink coloration indicates blood.

Luminol test: Luminol is a chemical which has been used to locate the bloodstains. The articles suspected to bear stains is sprayed with luminol. It reacts with blood to give fluorescence. The bloodstains are thus made visible. Even decomposed blood reacts with the reagent. The reagent does not interfere with subsequent blood tests. It is prepared by dissolving sodium perborate (0.7g) in water (100ml) and adding 3- arninophthalhydrazide (0.1g) and sodium carbonate (5.0g) to the solution. The solution is sprayed upon the article with glass sprayers in a dark room. Blood gives strong luminescence.

Crystal test: Two crystal tests are commonly employed. They are specific for blood but they are not sensitive. They often fail if the conditions are not rigidly controlled or if the blood is disintegrated or contaminated.

Teichmann Test: Take a dry crust or smear of blood on a slide. Put a drop of potassium iodide, bromide or chloride (0.1gm) solution in 100ml glacial acetic acid over the blood and cover it with a cover slip. Warm the slide gently till it gives out bubbles. Typically haemin crystals are observed under microscope. Heating may have to be repeated a number of times.

Takayama Test: It is also performed similarly. The reagent is prepared from one volume of glucose solution (10%), one volume of potassium hydroxide solution (10%) and two volumes of pyridine which are dissolved in six volumes of water.

11) Precipitin reaction The reaction is performed to identify the species of the origin of the bloodstains. It is very delicate test and requires only small amounts of blood. A dilute blood solution is used for precipitin reaction. The antisera are not diluted. The blood solution and antisera should be free from turbidity and contamination.

Blood stain patterns

The shape of the stain indicates the height from which the blood has fallen.

- Bloodstains from the height of upto 50 centimetres are round sharp edges.
- Bloodstains from a height of 50 to 150 centimetres have small spike like projections along the edges.
- Bloodstains fallen from a height over 150 centimetres have corrugated edges.

Testing of saliva

Saliva stains may be found at the scene, on handkerchief, on discarded cigarette stubs, spittal, on cups, tumblers, bottles on postage stamps or envelopes or even tooth picks or they may be found on a piece of cloth used as gag. Saliva contains an enzyme (ptyalin) which when added to starch, hydrolyses it. Saliva extract, therefore, when added to starch inhibits its color reaction with iodine. Saliva of secretors contains blood group substances and can be grouped. The saliva on cigarette stubs is often in criminal investigation. DNA profiling of the saliva stains has enhanced the evidential value of the saliva stains and has brought it at par with the other body fluids like that of blood and semen. Food material mixed in saliva may interfere with blood grouping. Saliva does not give specific precipitin test.

Forensic Analysis

Forensic Drug Analysis

In order to meet the legal requirements, the forensic analysis of such drugs proceeds from screening tests to more specific confirmatory tests. Suppose a white “Powdered Sugar” is obtained from the crime scene, the primary task would be to confirm the identity of the substance.

Most confirmatory analyses employed for drug identification are moderately time-consuming and require the use of many sophisticated and expensive instrumentation such as a gas chromatograph-mass spectrometer or a Fourier transform infrared spectrophotometer. To save time and money, before conducting a confirmatory analysis (potentially resulting in conclusive information), quick and inexpensive presumptive drug analyses should be done. These analyses not only direct the forensic scientist toward an appropriate confirmatory analysis that will yield the desired results but also saves time.

How drugs work

Depressants, such as barbiturates, glutethimide, chloral hydrate, methyprolon, and ethanol, have sedative/hypnotic properties that lead to anesthesia, coma, and death with increased dosage. Stimulants induce euphoria, a sense of well-being, increased mental activity, and anorexia in some cases. This class includes amphetamines, cocaine, caffeine, and phenmetrazine. Analgesics relieve pain. They may be narcotic and nonnarcotic and may be strongly addictive, mildly addictive, or not addictive. Psychomimetics create the perception of objects, sounds, smells, or sensations with no basis in reality. They include marijuana, LSD, and other hallucinogens. Cardiovascular drugs, such as diuretics and digoxin, as well as gastrointestinal drugs, like antiacids, may be less frequently abused. Chemotherapeutic agents include antibiotics, and they may be misused more often than abused.

Analysis of selected drug classes

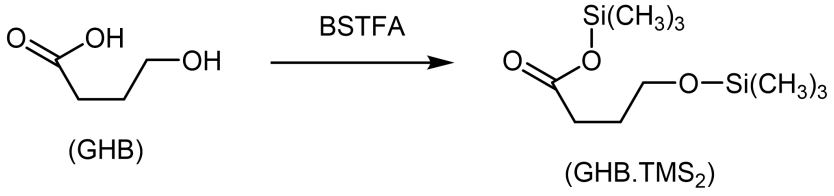
The forensic analysis of selected classes of drugs are explained below

Gamma Hydroxybutyric acid (GHB)

Gamma-Hydroxy-butyrate (GHB) is an endogenous metabolite found in most mammalian tissues at nanomolar concentrations. The sodium salt has been promoted as a steroid alternative as well as a tryptophan replacement. GHB often involves oral doses of the salt dissolved in water. GHB is a metabolic by-product of 1,4-Butanediol, a solvent used in the production of certain plastics and fibers, 1,4-Butanediol has also been used as a recreational substance. This application note describes a method for the analysis of GHB in serum, whole blood, vitreous fluid, urine, and tissue homogenates. A minimum of 0.5 mL of sample is required for the analysis. GHB is extracted from the biological samples using protein precipitation. The dried extracts are then derivitized with BSTFA prior to GCMS analysis. The MS data is acquired in a narrow scan range encompassing the masses of the specific derivative ions and qualifiers for GHB and GHB d-6 the internal standard.

Background

- The analysis of GHB in test samples with GC-MS or GC-FID may result in a false detection of the thermal decomposition product of GHB, Gamma-Butyrolactone (GBL). Legal classification of GHB is different than GBL, requiring the determination of GHB in a select way from GBL.
- To prevent the degradation of GHB at the sample inlet of the GC-MS or GC-FID, the chemical GHB is derivatized with N, O-Bistrifluoroacetamide (BSTFA). This derivatization results in a unique signal on the GC-MS and GC-FID that may be easily differentiated from GBL (SWGDRUG, 2005):



(GHB) (GHB.TMS$_2$)

- The molecular ion GHB-TMS2 with electron impact ionization (GC-MS) is weak at m/z 248, however, the signal at m/z 233 representing the methyl group cleavage is prominent.
- The chemical GBL does not derivatize with BSTFA and typically elutes earlier on a polydimethylsiloxane column.

- This method provides documentation for the examination of evidence to conform to the requirements of the Department of Forensic Sciences (DFS) Forensic Chemistry Unit (FCU) Quality Assurance Manual, the accreditation standards under ISO/IEC 17025 (current revision), and any supplemental standards.

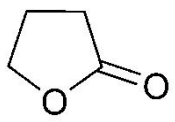
Procedure of analysis

- Place the test sample or standard (or extraction thereof) in a glass vial or test tube.
- Heat this sample or standard at about 55 °C for about 30min in a fume hood. \
- Add to this vial or test tube:
    - 100µL ethyl acetate (mix/vortex)
    - 100µL BSTFA (with or without 1% TMCS)
    - Cap immediately and mix/vortex
- Allow sample to incubate at room temperature for about 15 minutes.
- Transfer contents to GC-MS or GC-FID sample vial for analysis.

Limitations

- Although it is possible to simultaneously detect GBL with GHB via derivatization of GHB with BSTFA, caution should be used in interpreting GBL due to formation of GBL from excess GHB (i.e., non-derivatized GHB).
- BSTFA reacts with any protic solvent; thus, any source of aqueous solvents must be minimized. Use instead non-protic solvents, e.g., chloroform or ethyl acetate when possible.

Gamma Butyro Lactone (GBL)



amma-butyrolactone (GBL) is an industrial chemical, and is illicitly used as a substitute and chemical precursor to gamma-hydroxybutyric acid (GHB). GBL and 1,4-butadiene (BD) are structurally similar to GHB and there is evidence to confirm that GBL and BD are converted to GHB after oral administration. GHB is a schedule I depressant. GHB abuse became popular among teens and young adults at dance clubs and “raves” in the 1990s, and gained notoriety as a date rape drug.

GBL is a commonly used industrial chemical intermediate and solvent, which is found in paint removers, cleaners, adhesives, and nail polish removers. Worldwide production of GBL is measured in the hundreds of thousands of metric tons.

GBL has the molecular formula $C_4H_6O_2$ and the molecular weight 86.09 g/mol. It is a colorless, oily liquid with a bitter taste.

GBL is abused for its euphoric and sedative effects. GBL is mainly self-ingested, with an average recreational oral dose of 1 mL. GBL has a faster onset of effects and longer duration compared to GHB.

Pharmacology

GBL is readily converted into GHB by the body's own natural process. Because of this, GBL has similar pharmacological effects to GHB. GHB is present in the central nervous system in very small concentrations; it is a metabolite of the neurotransmitter gamma-aminobutyric acid (GABA). Scientific data suggest that GHB can function as a neurotransmitter or neuromodulator in the brain. It produces dose-dependent depressant effects similar to those of the barbiturates and methaqualone. Low doses of GBL/GHB produce drowsiness, nausea, and visual distortion.

At high doses, GBL/GHB overdose can result in unconsciousness, seizures, slowed heart rate, severe respiratory depression, decreased body temperature, vomiting, nausea, coma, or death. Sustained use of GBL/GHB can lead to addiction. Chronic abuse of GBL/GHB produces a withdrawal syndrome characterized by insomnia, anxiety, tremors, marked autonomic activation (i.e., increased heart rate and blood pressure) and occasional psychotic thoughts. Currently, there is no antidote available for GHB overdose.

Procedure of analysis

1. Samples in water

A color test is performed by placing a drop of liquid sample in a spot plate and adding 3 drops of GHB test reagent. A positive test will turn a greenish color and eventually will turn a pale blue. A negative test and a blank will stay bright orange.

The liquid sample can be prepared for FTIR analysis by placing an aliquot of the suspected sample in a mortar and drying it. Let the mortar cool completely before proceeding to FTIR analysis.

If an aqueous sample contains the lactone (GBL) instead of GHB, it will dry down to an oily residue instead of a powder. This oily residue may be analyzed on the FTIR and used to identify the GBL.

2. Samples in other aqueous medium

Extract 1 to 5 mL of the unknown sample three times with excess $CHCl_3$. Extract once more with an equal amount of $CHCl_3$. This will remove any residual GBL from the sample. Analyze the last $CHCl_3$ layer on a GC-MS to show that all the GBL has been removed. If GBL is still present, reextract and analyze the CHCl3 until no GBL is present.

If possible, dry down the extracted sample in a small (~10 mL) beaker. This can be accomplished by using a hair dryer; however, do not exceed 150°C or the GHB may convert to GBL.

Crystallize with acetone by adding 10 mL of acetone to the beaker containing the sample and heating it on a hot plate at about 70°C (the boiling point of acetone is 57°C) with stirring until it boils. This should take no longer than five minutes. Immediately pour the hot solvent into another small beaker and dry off the acetone using a hair dryer on low heat.

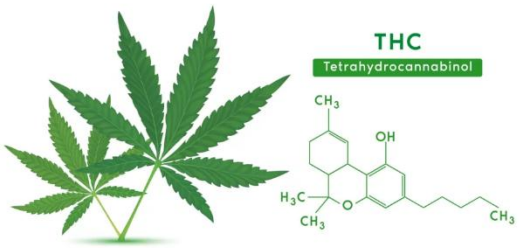
Add 2 mL of acetonitrile, 500 μL of BSTFA, and 800 μL of TCMS to the beaker containing the dry sample. Cover the beaker and heat the solution on a hot plate between 60°C and 80°C for 10-15 minutes. Do not exceed 80°C, and do not let the solution boil.

Analyze the product on a GC-MS. To identify GHB-TMS, ions 117, 133, 459, 204, and 233 must be present. Ions common to TCMS derivatives include 73, 147, and 243.

Marijuana

The identification of marijuana or its chemical constituents has long been one of the most often performed analyses in the forensic drug laboratory. This includes analysis of the very common botanical samples, ranging from whole plants to finely chopped vegetation, as well as preparations and extracts, such as hashish and liquid hashish. Analytical issues do not end

with merely identifying such exhibits. Occasionally the scientist is asked to compare exhibits to determine if they have a common provenance or what that provenance might be. This has resulted in a considerable body of literature devoted to profiling the constituents, both organic and inorganic, of Cannabis specimens Evidence of the use of Cannabis as a drug is also of forensic interest both in drugscreening programs and in cases in which drug-induced impairment is an issue. The focus in the analytical toxicology of Cannabis has been on the major metabolite 11-nor-Δ_9 - tetrahydrocannabinol carboxylic acid (THC-COOH). This chapter will address each of these topics separately and will include a historical perspective and cover specific widely accepted methodologies and recent advances.



Procedure of analysis

1. Fast Blue B Salt Test

a. Filter Paper Method

Preparation of reagent:

- Solid reagent : Dilute & mix Fast Blue B Salt with anhydrous Sodium Sulphate in the ratio of 1:100
- Solution 1 : Petroleum ether
- Solution 2 : A 10% w/w aqueous solution of Sodium Bicarbonate

Procedure:

Two filter papers are folded to form fluted funnels and kept on each other. Small amount of suspected sample is placed into the corner of the upper funnel of the paper and added two drops of Solution 1. Allow the liquid to penetrate to the lower filter paper funnel. Discard the upper filter paper and dry the lower filter paper. Now add a very small amount of the solid Fast Blue B reagent to this lower paper and add two drops of Solution 2. A purple–red coloured stain on the filter paper indicates the presence of cannabis product.

b. Test Tube method

Preparation of reagent:

- Solid reagent : Dilute & mix Fast Blue B salt with anhydrous Sodium Sulphate in the ratio of 2.5:100
- Solution 1 : Chloroform
- Solution 2 : 0.1N aqueous Sodium Hydroxide solution

Procedure:

Small amount of suspected material is taken in a test tube; a very small amount of the solid reagent and 1 ml of solution 1 is added to it. Shake well for one minute and add 1 ml of solution 2. Shake the test tube for two minutes, and allow this test tube to stand for 2 minutes. A purple red colour in the lower layer of chloroform indicates the positive result of the presence of cannabis product.

2. Duquenois-Levine Test

Preparation of reagent:

5 drops of Acetaldehyde and 0.4 gms of Vanillin are dissolved in 20 ml of 95% Ethanol.

Procedure:

Small amount of suspected material is taken in a test tube and shaken with 2 ml reagent for 1 minute, add 2 ml of conc. HCl and shake it well. Allowed it to stand for 10 minutes and then add 2 ml of chloroform. Appearance of violet colour in chloroform layer (lower layer) indicates the presence of cannabis.

3. Alternate Test

Preparation of reagent:

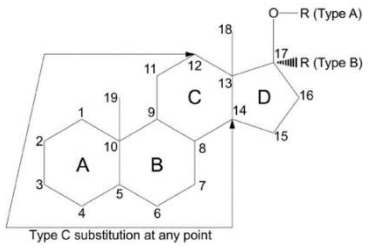
5 drops of acetaldehyde and 0.4gms of vanillin are dissolved in 20 ml of 95% ethanol.

Procedure:

The sample is extracted with petroleum ether. Filtered and evaporated to dryness. Added 2 ml. of Duquenois reagent to dissolve the residue add 2ml. Conc. HCl. Shaken and kept for 10min. Transferred the solution into a test tube add 2ml. of Chloroform and shaken. Purple colour in the chloroform layer indicates the Tetrahydrocannabinol.

Anabolic steroids

Anabolic steroids are synthetic derivatives of the natural male hormones Testosterone, which is produced principally by testes in males and is responsible for androgenic (masculinizing) and anabolic (tissue- building) effects. The abuse of anabolic steroids has historic foundations in man‟ s aspiration to create a body building "wonder drug". This group of drugs is responsible for the most pervasive abuse of drugs in sports. In human sport, anabolic steroids are used as bodybuilding drugs, occasionally in very large quantities, in events such as weightlifting and shot-putting.



There are generally two chemical types based upon the androstane and estrane ring systems: those with a 17**α**-alkyl group, which are active orally but possess hepatotoxicity, and 19-nor derivatives administered by injection. Although steroidal estrogens and some stilbenes reputedly possess anabolic activity and are engaged in beef production, they do not appear to be used as doping agents.

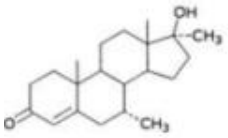
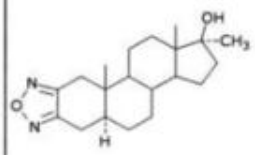
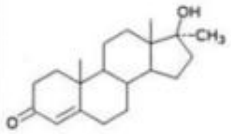
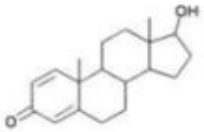
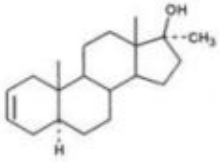
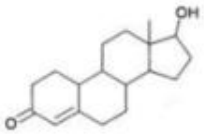
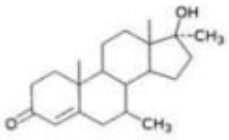
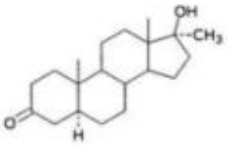
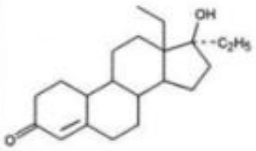
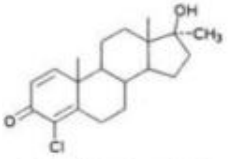
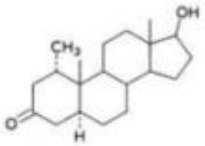
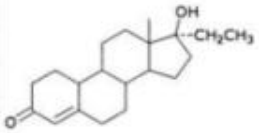
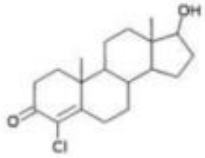
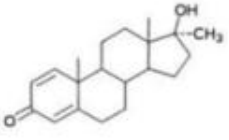
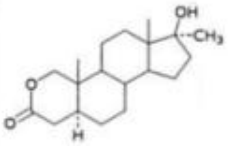
Anabolic Steroids reverse the negative or catabolic effects of exercise have on muscle. During exercise the adrenal glands naturally secrete glucocorticoids that induce muscle breakdown. Anabolic Steroids minimize this catabolism and thus build muscles.

Exogenous Anabolic Agents: Androstenediol, Androstenedione, Bolandiol, Bolasterone, Boldenone, Boldione, Calusterone, Clostebol, Danazol, Dehydrochlormethyltestosterone, Desoxymethyltestosterone, Drostanolone, Ethylestrenol, Fluoxymesterone, Formebolone, Furazabol, Gestrinone, 4- Hydroxytestosterone, Mestanolone, Mesterolone, Metenolone, Metandienone, Methandriol, Methasterone, Methyldienolone, Methyl-1-Testosterone, Methylnortestosterone, Methyltrienolone, Methyltestosterone, Mibolerone, Nandrolone, 19-Norandrostenedione, Norboletone, Norclostebol, Norethandrolone, Oxabolone, Oxandrolone, Oxymesterone, Oxymetholone, Prostanozol, Quinbolone, Stanozolol, Stenbolone, 1-

Testosterone, Tetrahydrogestrinone, Trenbolone and other substances with a similar chemical structure or similar biological effect(s)

Endogenous anabolic agents: Androstenediol, androstenedione, Dihydrotestosterone, Prasterone, Testosterone and metabolites and isomers

Other anabolic agents: Clenbuterol, Selective Androgen Receptor Modulators (SARMs), Tibolone, Zeranol, Zilpaterol

Parent	Trade Names (examples)	Parent	Trade Names (examples)	Parent	Trade Name (examples)
 Bolasterone	Myagen Methosarb	 Furazabol	Frazalon Miotolon	 Methyltestosterone	Android Metandren
 Boldenone	Boldane[1] Equipoise[1]	 Madol[7] (Desoxymethyltestosterone)		 Nandrolone	Deca-Durabolin[1] Anabolicus[3]
 Calusterone	Methosarb Riedemil	 Mestanolone	Andoron Notandron	 Norbolethone[7] (Genabol)	
 Chlorodehydromethyl-testosterone	Oral-Turanibol	 Mesterolone	Proviron Mestoranum	 Norethandrolone[7]	Nilevar
 Clostebol	Steranabol[2] Testɑnabol[2]	 Methandienone (methandrostenelone)	Dianabol Danabol	 Oxandrolone	Anavar Lonavar

Examples of commonly used anabolic steroids

Chemical syntheses of analogues of testosterone, related in structure, were undertaken by drug companies in an attempt to design steroids with enhanced anabolic effects compared to the androgenic effects. In this way, it was hoped that the anabolic effects could be harnessed for medicinal purposes whilst avoiding the undesirable androgenic effects that could cause masculinisation in women and children. Complete dissociation (separation) of anabolic from the androgenic effects was never achieved, however, and all the anabolic steroids developed have androgenic properties to varying degrees. Even so, anabolic-androgenic dissociation was considered to be sufficient to warrant many anabolic steroids being licensed as medicinal products in the 1960s and 1970s.

The efficacy of anabolic steroids in treating patients with considerable tissue (protein) breakdown has not proved convincing. By the end of the 1980s, most anabolic steroids had been withdrawn as licensed products in several countries, including the UK, although many anabolic steroids are still supplied legitimately in other countries. Anabolic steroids are now predominantly limited for the purposes of hormone replacement therapy in the testosterone-deficient male (hypogonadism), these being various formulations of testosterone and its esters, and some xenobiotic (foreign to the body) steroids for the treatment of specific diseases.

Steroids are sufficiently small to diffuse from the blood stream into cells, where they dock into receptors, which are much larger specialised molecules. There are different receptors for each class of steroids in target tissues, that is, there are specific receptors for androgens, oestrogens (female sex hormones), glucocorticoids (helping to regulate blood glucose levels and also the body's overall response to stress) and mineralocorticoids (regulate salt balance). Androgen receptors are present in cells in reproductive tissues, skeletal and heart muscle, bone, hair follicles in the skin, liver, kidney and brain.

Anabolic steroids activate their receptors through complex molecular mechanisms to cause changes associated with androgenisation and effects on muscle. Usually, the body has a natural balance between growth (anabolism) and breakdown (catabolism) of cells. However, if testosterone is administered chronically in doses sufficient to considerably raise testosterone in the blood circulation, this causes a swing towards anabolism. For example, there will be growth of skeletal muscle and an increase in strength and power, which can also be enhanced by taking exercise. Other androgenic effects also are augmented by exercise.

Procedure of analysis

Infrared spectroscopy analysis

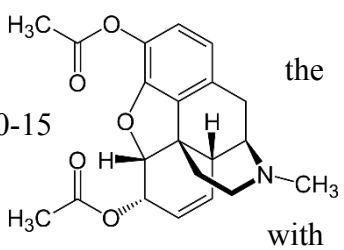
One drop of each formulation should be placed into the equipment and measurement should be conducted in scan mode from 4000 cm^{-1} to 600 cm^{-1} with a resolution of 4 cm^{-1}. After each analysis, the equipment should be cleaned with acetone and a blank measurement should be made.

Extraction and GC-MS analysis

Extraction of AASs should be performed using UAE and MAE. For UAE, 25 µL of the formulation and 5 mL of methanol should be placed in conical tubes and vortexed for 10 s. Subsequently, samples should be immerged in an ultrasonic bath at room temperature for 10 min and centrifuged at 3000 rpm for 5 min. Finally, the methanolic fraction should be transferred to vials and injected in the GC-MS.

For MAE, 25 µL of the formulation and 5 mL of methanol should be placed in a round-bottom flask and mixed for 10 s. The sample should be introduced into the microwave equipment and subjected to 150 W of power for 10 min. Afterwards, the methanolic portion should be transferred to vials and analyzed by GC-MS.

Heroin



Heroin (diacetylmorphine) is a white crystalline powder, which is derivative by adding two acetyl groups to the morphine, found in the opium. Heroin in impure form is known as Brown Sugar. It is 10-15 times more effective than morphine. It may be either injected or sniffed to cause similar effects as that of opium and heroin but with higher magnitude.

Procedure of analysis

Bases

Heroin base is soluble in carbon tetrachloride, but all known salts of heroin are completely insoluble. Morphine base is insoluble in water, and has some slight solubility in benzene and chloroform.

Hydrochloride salts

Heroin hydrochloride is soluble in chloroform and methylene chloride. Heroin tartrate, heroin citrate, and most inorganic chlorides are insoluble in these solvents. Morphine salts are essentially insoluble in chloroform and benzene, while the base has some solubility in both solvents. When a water soluble chloride is treated with silver nitrate solution a white precipitate forms. The precipitate is insoluble in concentrated nitric acid, and after washing the precipitate with water, is soluble in dilute ammonia solution, from which it can be re-precipitated by the addition of nitric acid.

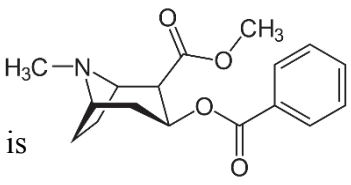
Tartarate salts

Heroin tartrate is insoluble in methylene chloride or chloroform, but is soluble in methanol. Morphine tartrate is water soluble while morphine acid tartrate is only slightly soluble in water. Silver nitrate will produce a white precipitate when mixed with a water solution containing either free tartaric acid or a tartrate salt. The precipitate is soluble in nitric acid. Congo red will produce a negative result with tartrate salts, but forms a blue colour when the free acid is present (congo red will also give a positive result if salicylic acid is present).

Citrate salts

Heroin citrate is insoluble in methylene chloride or chloroform, but is soluble in methanol. Silver nitrate will produce a white precipitate when mixed with a water solution containing either free citric acid or a citrate salt. The precipitate is soluble in nitric acid. Acetic anhydride can be used to test for citrates and free citric acid. The test involves the addition of 0.5 ml acetic anhydride to a small amount of sample in a test tube, and heating the tube at 80°C for 10 minutes. A purple colour will develop if a citrate salt or free citric acid are present along with a tertiary amine, e.g., heroin.

Cocaine



Cocaine is also a potent stimulant that produces similar effects as that of amphetamines- namely, increased alertness accompanied by suppression of hunger and fatigue. It is generally sniffed and is absorbed into the body by the mucous membrane of the nose.

One other form of cocaine which is quite popular is “crack”. It is manufactured by heating the mixture of cocaine, baking soda and water. It is also snorted and produces similar effects like cocaine.

Procedure of analysis

Colour reactions are produced by compounds with a particular chemical structure. The colour obtained in any particular test may vary depending on the conditions of the test, amount of substance present and extraneous material in the test sample. Colour test reagents must be checked with known substances when prepared. A blank should be run to preclude false positive results.

It must be stressed that positive results to colour tests are only a presumptive indication of the possible presence of cocaine. The colour tests for cocaine are especially prone to produce false positives. Many other materials, often harmless and uncontrolled by national legislation or international treaties, may give similar colours with the test reagents. A number of these are either other controlled drugs, often encountered as white powders (e.g. methaqualone), or the synthetic local anaesthetics which are often substituted for cocaine in the illicit traffic. It is mandatory for analysts to confirm such results by the use of alternative techniques.

The colour test described below is known as the Scott’s Test (modified Cobalt Thiocyanate Test).

Reagent 1: Dissolve 1.0 g cobalt thiocyanate in 50 ml of 10% (vol/vol) acetic acid, then add 50 ml of glycerine

Reagent 2: Hydrochloric acid (concentrated)

Reagent 3: Chloroform

Method:

Step 1: Place a small amount (no more than 1 mg) of the suspected material in a test tube. Add 5 drops of Reagent 1 and shake the test tube for 10 seconds. Cocaine and related substances produce a blue precipitate and a blue solution.

Step 2: Add a drop of Reagent 2 and shake the mixture for a few seconds. The blue solution should turn pink. If the blue colour still does not change, add one further drop. If still no change, repeat the test with a smaller portion of suspected material.

Step 3: Add 5 drops of Reagent 3 and shake. If cocaine is present, the lower chloroform layer will develop an intense blue colour, while the upper layer will be pink.

Results:

A positive result at each stage is required in order to be considered as a positive test for cocaine. Only a few non-controlled or controlled drugs will give a similar colour sequence.

Microcrystal tests

Microcrystal tests are quick, simple and extremely sensitive test for the identification of substances. They involve the formation of crystals from the reaction of the target compound with a chemical reagent, followed by the analysis of the resulting crystals by means of a polarizing microscope and comparison with reference material.

Platinic chloride test

Reagent: Dissolve 1 g of platinic chloride in 20 ml of distilled water.

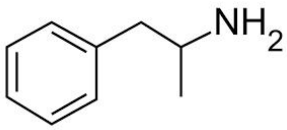
Method:

Place 2 drops of sample solution (approximately 2-3 mg of the sample/5 drops of 10% hydrochloric acid) on a clean microscope slide. Then place 2 drops of the reagent near the drops of the sample and use a glass rod to create a tiny channel connecting the solutions. Observe the reaction and resulting crystals without a cover slip at 100-200 magnification using a polarized microscope.

Results:

Cocaine forms V-shaped long, thin needles with branching.

Amphetamines



Amphetamine or its derivative methamphetamine may be injected direct into the blood stream through intravenous injection. The desire for a more intense experience is the chief motive behind this route of administration. First sensation of "flash" or "rush" initiates, followed by high feeling of pleasure, euphoria that produces hyperactivity, with a feeling of clarity of vision as well as hallucinations. After the effect wears off, the individual passes through a period of exhaustion and may sleep continuously for one to two days.

Another type of amphetamine is methamphetamine called "speed" because of its rapid stimulation of central nervous system.

Procedure of analysis

Marquis Reagent test

Preparation of reagent: To the 1ml of Formaldehyde solution, few drops of conc. Sulphuric Acid is added and stock solution is prepared.

Procedure:

- Few drops of sample are placed in spot plate.
- Then 2-3 drops of stock solution is poured on the plated

Result : Orange- Red Colour for both amphetamine and methamphetamine.

Simon's Reagent test

Preparation of reagent:

Solution A: 20% aqueous Sodium Carbonate solution.

Solution B: 50% ethanolic Acetaldehyde solution.

Solution C: 1% aqueous Sodium Nitroprusside solution.

Procedure:

- Appropriate amount of sample is taken on spot plate
- A drop of Solution A is added followed by one drop of Solution B.
- Further, few drops of Solution C is added.

Result: Appearance of blue colour indicates the positive tests for the presence of methamphetamine while appearance of slow pink to cherry red colour indicates the presence of amphetamine.

Libermann's test:

Preparation of reagent: 5 gram of Sodium Nitrite is added in 50 ml. of Sulphuric Acid.

Procedure:

To the sample, reagent is added drop wise.

Result: Red- Orange color indicates the presence of Amphetamines.

Mandelin's Reagent Test

Preparation of reagent: 1 gram Ammonium Vanadate is dissolved in 100ml. concentrated Sulfuric Acid.

Procedure:

- Appropriate amount of aqueous solution is taken.
- Few drops of Mandelin's reagent is added

Result: Appearance of green color darkens rapidly indicates the positive test for the presence of amphetamines. On stirring the colour passes through several shades to emerald green and dark reddish brown, which changes to light red-brown on heating.

Forensic analysis of Inks and Paints

The liquid that carries the colorant water is referred to as aqueous inks. Water based inks come in two varieties that is dye and pigment. Dye ink is water dissolved in colour as they are cordials and are best in use. The colorant which is used in dye have a tiny size and allow a very small dot size which allows detailed images with smooth tone. Dye inks will fade in UV light and if water is poured onto a dye the colorant dissolves in the water again and washes away or runs. So dye inks are not waterproof.

Properties of Ink

- Ballpoint ink is specially formulated to be thick and fast-drying. Its viscosity is precisely controlled: it must be as thick as possible but still thin enough to flow down the barrel of the pen in response to gravity.
- Ballpoint pen inks use dye because the tiny particles of undissolved pigment can clog the ball of the pen.
- Ballpoint pen ink is usually oil-based to give it its thickness. Oil also is why ballpoint pen ink dries quickly and is permanent and water-fast.
- Fountain pen ink is a water based ink (dye) intended for use with fountain pens. Red ink usually contains the eosin dye where as blue ink often contains triarylmethane dye.
- Gel pen ink is a pigment suspended (colloidal) in water based gel and it is opaque and thick in appearance.

Forensic analysis of ink

- The forensic analysis of ink is a frequent requirement in casework in order to identify whether the inks on a document or documents are same or different.
- Hence, the document examiners are often called upon to identify the ink involved and to determine the actual source of the ink on a document.
- It’s a crucial part of forensic document examination since it is used in both handwritten and printed documents.
- The importance of ink analysis has been noted in case of obliterations for differentiating the ink on basis of its chemical composition; further, the source and also the age of ink which ultimately gives the approximate age of the document providing leads and clues in the investigation process.

- There are different types of inks available which are used in different pens such as ball pen ink composing of dyes and organic solvents, gel pen, roller ball pen which uses gel-based inks, fiber or porous tip pen, fountain pens that uses water-based inks etc

Ink Analysis Methods Used in Questioned Document Examination

Non-Destructive

Integrity of the sample having evidential importance is of fundamental value in forensic examination. Hence, in order to maintain the integrity of documents, the non- destructive techniques are always preferred over the destructive ones.

Microscopy

- Preliminary examination of ink is performed prior to the chemical and instrumental analysis. This includes examination of ink by the use of microscopes and optical sources without detaching the inked portion from the document.
- The stereomicroscope is used to determine the type of ink, its color or to determine additional marks generated due to pointed tip, blunt tip, damaged writing tip leading to the identification of a class of writing instrument.
- This helps in determining the composition of ink used. In optical examination various light sources like UV radiations, IR radiations are used as different inks reflect differently with these light radiations.

Video Spectral Analysis of Ink

- We are aware about the fact that the unaided human eye can perceive only a certain range of colors of a particular wavelength in the electromagnetic spectrum.
- Yet there are other ranges also present such as infra red and ultra violet range. Although these are invisible but can be converted to visible using instrumentation.
- VSC is that one comprehensive digital system which aids our eyes to perceive these invisible ranges.
- Inks are composed of different dyes. These dyes or the colors can appear similar to our unaided eyes. For example, two blue colored inks are difficult to be differentiated by visual observation as the color is same but the colors could behave differently when perceived under UV or IR range.

- It’s this behavior that lets the examiner differentiate it. The specialized filters of light and the photographic films allow uncovering the minute details of the document sample.
- These aids show peculiar properties such as luminescence, transparency, etc. depending upon the chemical properties. Thus, the VSC is one of the reliable instruments used for the questioned document examination.

Destructive

Techniques incorporating chemical analysis of the ink present on the document fall under destructive category since for sample analysis the portion of the document containing ink is cut and thus the sample is destructed.

Chromatography

- Various chromatographic techniques such as TLC, GLC, and HPLC are utilized for the qualitative ink analysis in order to separate the components of ink thereby determining the composition.
- Most commonly used technique is the TLC (Thin Layer Chromatography) which incorporates the use of chromatographic plate and the alcohol based solvent for the purpose of ink separation.

Capillary Electrophoresis

It is similar to HPLC but uses a small amount of samples. Hence, adopted in cases where the sample is limited. Herein, the capillary is filled with a conductive fluid with a designated pH value. The conductive fluid then serves as the buffer solution in which the sample is separated.

Infrared spectroscopy

It is used for the characterization of ink. The dyes, additives and solvent present in the ink show different absorption peak in the spectrum which are unique for every substance and compared with the standard peaks for identification purpose.

GCMS

- GC along with MS i.e. mass spectrometer is one of the best techniques used for ink analysis wherein firstly the components are separated by gas chromatography followed by the identification on the basis of mass/charge ratio.
- In the examination of questioned documents, the non-destructive techniques are preferred until and unless it is very necessary to adopt the destructive ones in order to reach to a definite conclusion.

Questioned documents

Wherever question over the authenticity of any document is raised, the questioned document examiners are called upon.

Questioned document is basically a document having authenticity under question which is subjected to a forensic document examination.

The primary purpose of examining the disputed documents is to give opinion upon the questions that are raised upon the authenticity of the document using scientific principles and techniques.

Questioned documents could involve any type of document for an instance a property will, a suicide note, a blank sheet possessing indentations, bank cheques and many more.

The examination of documents includes detecting alterations and obliterations, analysis of paper, detection and examination of forgery, origin, determining authenticity, and others.

This blog summarizes ink analysis methods utilized in forensic questioned document examination as well as the importance of ink analysis so far in the field of investigation.

What is The Role of Forensic QDE (Questioned Document Examiner)?

The forensic document examiner plays a crucial role of uncovering the minute details in the document sample such as in signature or in handwriting. These are the details that cannot be detected or uncovered by an untrained person.

Physical analysis of ink and paper

An examination of a disputed document is a scientific problem and it is no longer confined to comparison of signature or writing but it also includes examination of ink, typed matter, printed matter, watermark and all other elements that combine together to form a document. The examination of a document is not complete until its every element has been studied and examined thoroughly.

Points which are generally considered in the preliminary examination:

- Is the paper of unusual size and was it cut or trimmed by hand at any margin?
- How many times, the paper has been folded and what is the sequence of folds?
- Does the paper bear any marks of artificial ageing and has the paper been torn, wetted, mutilated in any way and if so, then what are the probable reasons?
- Does the paper bear indentation marks on the front side or embossed strokes on the reverse side?
- Does the paper show any physical or chemical erasing, obliteration, substitution or alteration of any kind?
- Was the document written or typed before or after the paper was folded?
- Was more than one kind of ink or pens used to write the body writing of the document?
- Does the document show any unnatural spacing between the written or typed lines?
- Is the body writing of document written in continuity by one and the same writer?

Any unusual feature, etc. Forgery owes it origin to the inborn tendency in human beings to imitate, to which we may trace all arts, fines or useful. Its detection is possible through another trait known as inherent quality of imperfection attending all human acts and performance. The ‘counterfeit’ looks alike but doesn’t reach upto the genuine. It would be more true to say that nature never repeats itself than to assert the contrary.

Physical examination

This includes the optical examination of ink with the help of hand magnifier or compound microscope to determine:

- The type of ink used(ball point pen, fountain pen or fiber tip etc)
- Color of ink

- Comparison of secondary color shades, ultraviolet rays are used to compare the degree of fluorescence.
- Infrared rays are used to differentiate dyes and pigments and especially ball point pen inks

Chemical analysis ink and paper

If two inks are found same from the physical examination then chemical examination can be avoided, because test results in the alteration of at least some part of the document. The chemical analysis of ink can be conducted in two different ways

1. Performing chemical spot tests on the punched out fragments of ink strokes or on the ink strokes itself.

2. Chromatographic analysis for isolation and characterizing various dyestuff inks.

Prior to advance techniques the ink were chemically analysed and differentiated by chemical spot tests, preformed on the ink itself or on small fragments of ink strokes, punched out from the ink line with the help of scalpel hypodermic needle or punching plate etc. an now these chemical spot test are exchanged by the thin layer chromatography and other sophisticated techniques. However, some cases, the spot test may prove useful for conducting preliminary examination. Numerous reagents have been suggested by different workers for performing spot tests. The following reagents may serve the purpose.

1. Bromine water : saturated aqueous solution
2. Sodium hydroxide : 2N aqueous solution
3. Hydrochloric acid : 2N aqueous solution
4. Stannous chloride : 10% aqueous solution
5. Sodium hypochlorite : saturated aqueous solution
6. Oxalic acid : 5% aqueous solution

Carbon inks can be distinguished from other writing ink as this ink remains unaltered by the action of reagents.

Logwood ink and iron-nutgall inks can be differentiated with hydrochloric acid test. The logwood inks with these reagents give red or purple – red colour reaction, whereas the iron-nutgall inks give blue or blue green reaction.

Nigrocine inks can be differentiated from the logwood and iron nutgall inks with oxalic acid test. The oxalic acid has little effect on nigrocine, whereas this reagent bleaches the iron nut-gall inks to some extent or it may change colour of logwood and iron nut-gall inks.

Analytical methods

Gas Chromatograph–Quadrupole Mass Spectrometer (GC–QMS) and GC– MS–MS

The GC–QMS is also called as GC–mass selective detector or GC–MS, commonly that requires the analytes are chemically extracted from blood, urine, or other matrices and, in most cases, derivatization technique is used to make them volatile and prior to introduction into the instrument. The GC separates the compounds based on differences in solubility and volatility in the liquid, solid, and gaseous phases. Molecules enter into the ion source sequentially, they are ionized. Electron ionization (EI) is the most common ionization technique. Molecules leaving the GC enter the QMS and are bombarded by a beam of electrons. Electrons are removed from the molecules, producing unstable positive ions (molecular ions) which break into more stable fragments. Chemical ionization (CI) is a "soft ionization" technique utilizes a charged reagent gas (NH_4 or CH_4) to transfer charge to a compound. These charged species are more stable than the EI ions and fragments are formed less extensively. Both positive and negatively charged molecules are formed. The QMS will select ions and measure their abundance from different mass-to-charge (m/z) ratios. Specific ions are isolated by creating a dynamic electromagnetic field inside the quadrupole. The QMS can be configured to scan all the mass-to-charge ratios in its mass range and track specific mass-to-charge ratios. In forensic toxicology laboratories, GC–MS–MS procedures are sufficiently more common following advances in vacuum engineering, ion sources, instrument size and operating software. GC–MS–MS procedures were improved for measuring low concentration of analytes (e.g., LSD) in blood or urine. Current GC–MS–MS methods improve S / N in essential experiments with LODs of 0.1 ppb (5 pg on column) including examination of metabolites of the nerve agent and sulfur mustard in urine and metabolites in alternative matrices.

Raman Spectroscopy Techniques

Raman spectroscopy is one technique used in forensic sciences. This method allows the measurement of inelastic light dispersion due to the molecular vibration modes when irradiated by a monochromatic source, such as a laser. The Raman technique has many benefits, such as its nondestructive nature, its rapid analytical time and the possibility of performing microscopic in situ analysis. This is a multi-purpose technique in its forensic application that covers a broad variety of samples, such as drugs of abuse, physical / trace evidence, fibers and inks. The analysis of textile fibers constitutes a clear example. In this field a number of studies have been carried out. The value of this technique in forensic analysis of fibers mainly focuses on the detection of dyes.

Atomic Absorption Spectroscopy

Hair analysis is done by Atomic Absorption Spectroscopy after sodium hydroxide and hydride generation technique. Many of the arsenic particles on the hair have also been measured in KEKPF's BL-4A (Photon Factory at High Energy Accelerator Research Organization, a synchrotron radiation facility in Tsukuba) and found an arsenic particle on the hair. This method would allow the measurement of inelastic light dispersion due to molecular vibration modes when irradiated by a monochromatic source such as a laser. The size of the beam of synchrotron radiation along with the hair shaft was 4 or 1 mm in diameter. How many hair shafts were counted, and how many particles were found, is still not clear. It is said that arsenic particle was found on only one or two hair shaft out off hundreds of hair shafts.

LC tandem Mass Analysis

The tandem Mass spectrometer is generally used an electrospray ionization source in a positive mode. The chromatograms are separated by using suitable columns. At room temperature, mobile phase Solvent A consisted of 5% acetonitrile with 0.1% formic acid and Solvent B consisted of 95% acetonitrile with 0.1% formic acid. The chromatography system is operated at gradient mode by changing the solvent ratios at different time intervals. The urine samples are generally stored at 200C until analysis. Take 1mL urine sample and add 0.5mL 1.5M sodium bicarbonate buffer (pH 9.5) and 3mL ethyl acetate for liquid-liquid extraction. The samples are mixed on a suspension mixer, centrifuge for 3 minutes and collect the supernatants were decanted, dried under nitrogen gas. The residues were dissolved in

0.5mL of 5% acetonitrile with 0.1% formic acid. The mixture was vortex for 10 seconds and filtered through a 0.22µm polyvinylidenedifluoride filter into a small volume of auto sampler vial. Then, 50µL was injected into the LC-MS/MS system. All samples are prepared in this same manner

Optical microscopy

Optical or light microscopes are highly crucial instruments that use light as a probe in order to determine the structure and morphology of a particular compound. Light as such is an electromagnetic radiation in which the two components that are primarily present—the electric field and the magnetic field—oscillate at right angles to each other. Light itself is composed of photons that contribute the particulate and the wave-like nature to light. Due to such properties, parameters such as amplitude that determines the height of the oscillation, wavelength, frequency, and the energy of light are manipulated in order to obtain a higher-resolution image of the sample. There are mainly two types of microscopes: simple and compound. While the simple microscope is composed of a single lens and is simply used to visually observe the samples, the compound microscope itself is made up of two or more lenses and a light source. Based on the type of light source and the way a sample is viewed, the compound microscopes are divided into various types.

In forensic science, light microscopes are often one of the earliest tools used for the preliminary and confirmatory analysis of evidence. There is a myriad of evidence found and each sample has its own method of preparation and viewing. In this chapter, we present a brief overview on the different types of optical microscopes, namely the phase contrast, bright field, confocal, fluorescence, polarized, and stereomicroscope.

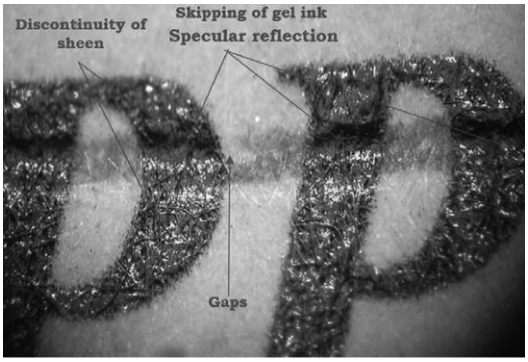
Paints

Paint analysis is usually performed in cases related to hit and run, or for the samples collected from tools involved in a burglary. Microscopic analysis reveals the layers of paint, by which it can be correlated to the vehicle or source from where it came. This analysis is commonly practiced in the forensic examination of vehicles. Hamer published an article in the early 1980s regarding the use of light microscopy along with plane polar and crossed polar lights for the forensic analysis of pigments present in paints. The images were recorded in the magnification range of 150 to 625. Properties such as color, shape, size, crystallinity, and

refractive index of paint samples were analyzed. Allen also used light microscopy for the investigation of thin sections of household paint samples. The illumination type was both bright-field and dark-field. These microscopic techniques helped in the discrimination of paint samples on the basis of their color as well as particle distribution. In addition, PLM was used for analyzing the birefringence and fluorescence microscopy for the fluorescent property of the paints. In another study that focused on the investigation of architectural paints, a stereomicroscope was used for the analysis and discrimination of such paint samples on the basis of properties such as topcoat sheen and color as well as the layer color, texture, number, and thickness. The microscopic investigation helped in discriminating paints on the basis of their topcoat as well as the structure of layers they had.

Questionable documents

Questionable documents are analyzed in cases of counterfeiting or forgery. Chromatographic analysis usually helps in the chemical profiling of inks while microscopic analysis helps in the examinations of line crossings. In this regard, Saini et al. used stereomicroscopy for the analysis of line crossings through gel pens and strokes of a laser printer. The magnification used for capturing the images was in the range of 2.5 to 40 while the illumination angles for the light source were 15 and 45 degrees. Features such as the sheen, gap, and skipping of the ink as well as specular reflection were visualized. Figure below shows the sheen discontinuity, gel ink skipping and gaps, and specular reflection in a writing sample.



Fluorescent techniques

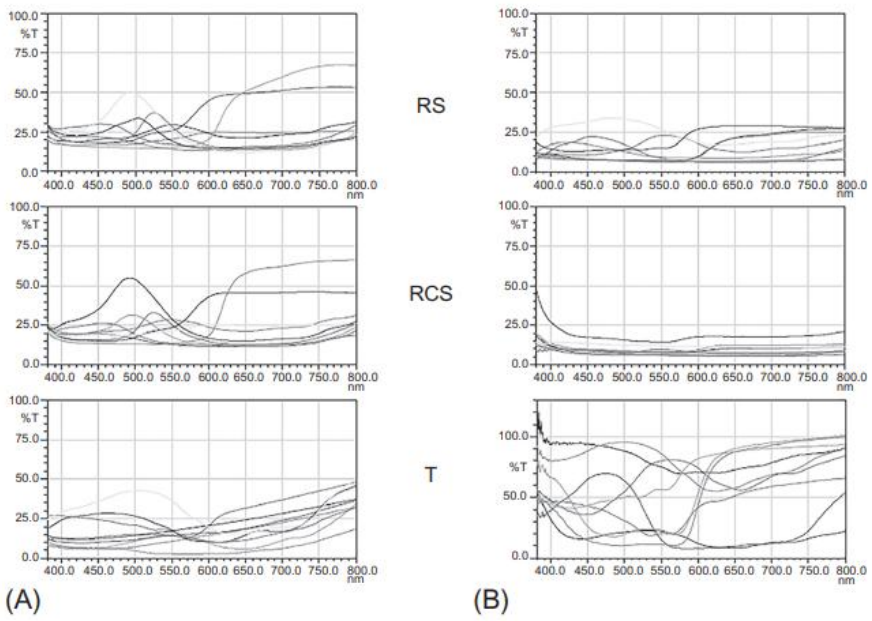
The basis of this technique is the photonic processes that are involved in the electronic transitions from a ground state to the excited state. As mentioned in the previous section, when the energy of the photon matches the energy of each molecular level, then the electrons

get excited and move to a higher level. However, it is important to consider that each molecular level or electronic state is further subdivided into a number of subvibrational levels that are separated by a distance of 100 cm^{-1}. Due to this, the actual distance between the ground state of the electron and the excited state of the electron is significantly higher, thus promoting the preference of the electron to rest in its ground state. Similar to a chromophore, a fluorophore is responsible for emitting energies with wavelengths longer than the excitation energy during the relaxation of the excited electron to the ground state. These molecules get excited when the polarization of the incident light is aligned along with the excitation dipole of the molecule, thus resulting in fluorescence.

Once excited, the electrons undergo two main types of decay to return to the ground state: radioactive and nonradioactive decay. In radioactive decay, photon emission occurs while in nonradioactive decay, photon emission does not occur. The fluorescence lifetime of a molecule is therefore measured as the combined rate of the radioactive and nonradioactive emissions.

Paints

Paint analysis has forensic importance in cases related to hit and run or vandalism. Different types of techniques are employed to study the morphological and chemical features of paint and its layers. Visible spectroscopy has also been utilized in some studies. In a study, visible spectroscopy was used for the forensic investigation of car paints. Paint chips of blue, red, and green were observed through light in a perpendicular manner at the cross-section as well as at the top layer of the paint fragment. The analysis was carried out in the transmittance and reflectance modes. It was observed that the spectra showed greater variation for different colors when the top layer analysis was done. Figure below shows the absorption spectra of car paints. In another study, visible spectroscopy extended to the near-infrared region was used to forensically analyze and distinguish different car paints. The analysis was carried out in the range of 400–1100 nm.



Visible spectra in reflectance (RS and RCS) and transmittance (T) mode for (A) solid; and (B) metallic car paints

Questionable documents

Documents become forensically important in cases related to forgery. The inks used in such questionable documents as well as line crossings and fibers are analyzed using different characterization tools. UV-visible and fluorescence spectroscopy have also assisted in this regard, mostly in the investigation of inks used in questionable documents.

In a study by Vogt et al., fountain pen inks were studied using capillary electrophoresis embedded with fluorescence and a UV-visible detection method. The spectra (UV-visible and fluorescence) of ink samples collected at variable λ_{ex} and λ_{em} were compared with those of standard ink compositions in order to identify them. In another study, ballpoint pen inks of blue were discriminated on the basis of their visible spectra, which was recorded in the range of 400–750 nm. It was observed that inks of different brands had different overall absorbance spectra, with their maxima ranging from 550 to 700 nm. Causin et al. used absorption spectroscopy in the range of the UV, visible, and near-infrared region for the forensic examination of paper. The spectra were recorded in the range of 200–900 nm. Different brands of paper were differentiated on the basis of their average reflectivity in the 680–900 nm range as well as their maxima in the 272–360 nm range. In another study, black inkjet inks used for printing documents were analyzed using visible spectra extended to the near-

infrared region. The spectra were recorded from 500 to 1000 nm. It was observed that inks of different brands had different absorption spectra in the 600–1000 nm range.

TLC

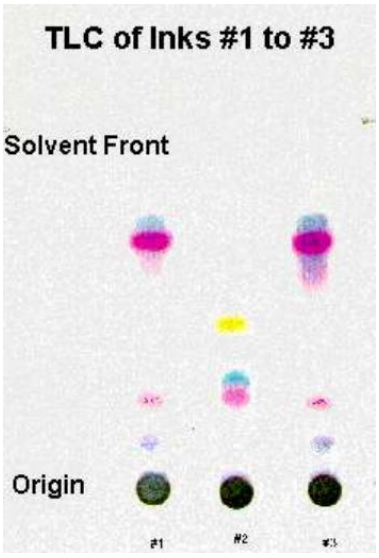
Thin layer chromatography (TLC) is considered to be the most suitable technique for isolating and identifying various components of inks. In these techniques a thin layer plate is prepared by coating a glass plate with silica get or aluminum oxide but the readymade silica gel G plates are available in plate. Depending on the amount of ink deposited on the paper, 1 to 10 plugs of ink are removed with the help of a spatula or hypodermic needle and the ink is dissolved in the minimum quantity of a suitable solvents. A few micro liters of the solution are spotted with a capillary tube onto the layer on the thin layer plate and plate is then placed in a closed jar having selected solvents in fixed ratios. The liquid slowly begins to rise up the plate and when it moves past the sample spot, the components of the sample get separated and get located at different heights. When the liquid phase has moved a sufficient distance the plate is removed from the jar and the Rf value of different spot are recorded.

For differentiation of two inks, their spots are marked on the same TLC plate and if these samples show same number of spots with the same Rf values and colours, then the two inks are identical in their dyes composition otherwise not.

Rf value is defined as the distance travelled by the component divided by the distance travelled by the liquid moving phase.

Different solvent systems for ink analysis:

1. Butanol: ethanol: water – 50:15:10
2. Ethyl acetate: cyclohexane: methanol: ammonia – 70:15:10:5
3. Ethyl acetate: butanol: ammonia – 50:35:5
4. Ethyl acetate: ethanol: water – 70:35:30
5. Toluene: acetate: ethanol: water – 30:60:7:2



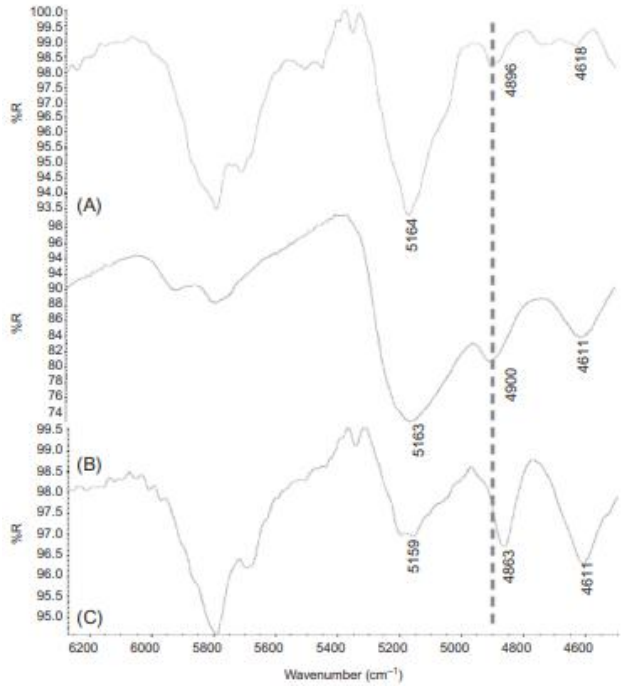
The band patterns corresponding to ink removed from three different areas of a document (figure) show that samples #1 and #3 have a similar formulation while ink #2 contains different components than the other two

FT - IR

Paint analysis is of great forensic importance when it comes to investigations related to hit and run or vandalism. As per the Locard exchange principle, paint traces are exchanged whenever any vehicle hits an object or person, or any tool is smashed on any wall or object. Such cases require the analysis of paint by which the origin of that paint source, its manufacturer, etc., can be identified. This section focuses on the research works that have utilized FTIR/NIR spectroscopic techniques for the examination of paints.

In a study by Muehlethaler et al., FTIR in combination with Raman spectroscopy and chemometrics was used to forensically analyze paint samples. A total of 34 red paint specimens were collected and analyzed through FTIR spectroscopy, in which the spectra was recorded for the spectral range of 4000–650 cm^{-1} with a resolution of 4 cm^{-1}. The composition of paints as per the IR spectra of seven groups formed was comprised of binders such as acrylic and styrene as well as extenders such as magnesium silicate and calcium carbonate. Another group led by Muehlethaler published a research work focusing on the use of FTIR spectroscopy for the discrimination of spray paints of different colors such as green, blue, and red. A total of 74 cans of spray paint was used for the study. The IR spectra were obtained from 4000 to 650 cm^{-1} at 4 cm^{-1} resolution. It was observed that alkyd binders (orthophthalic

acid) were commonly used in paint cans collected from the market as compared to the acrylic binders. The study proposed developing a database of the FTIR analysis of paint samples that can assist crime investigators in solving cases. In another study by Sciutto et al., FT-NIR was used as an advanced spectroscopic technique for the examination of crosssections of paint samples. The IR spectra were recorded in the NIR and MIR region in the range of 8000–675 cm^{-1}, having a mode of total reflection and a resolution of 4 cm^{-1}. Figure given below shows the NIR spectra of the first layer of paint sample and reference NIR spectra for animal glue and egg protein. The spectra of paint matched with that of animal glue at 4896 cm^{-1}, suggesting the presence of animal glue in the paint sample.



IR spectra of (A) first layer of paint sample; (B) animal glue; (C) white egg protein

Forensic Toxicology

Forensic Toxicology

The word “Toxicology” is came from the Greek word “toxicon” which was used as a poisonous substance in arrowheads. Conventionally, the toxicology may be defined as the science representing the character, source, knowledge, lethal dose, fatal effect, analysis of poisons and their curative measures. More precisely, Toxicology can be said as the study of antagonistic effects of chemical or physical agents on living organisms. A “toxicologist” is competent to examine and lead into the nature of those effects on human, animal, and environmental health. Toxicological study examines the cellular, biochemical, and molecular mechanisms of action as well as functional effects such as neurobehavioral and immunological, and assesses the likelihood of their event.

The diversity of chemicals and that of their prospective adverse effects in the environment make toxicology a comprehensive science, which often demands specialization in a specific range of toxicology. Our society’s dependency on chemicals and the requirement to assess impending hazards have made toxicologists an increasingly important part of the decisionmaking processes. The existing description would be somewhat altered but not necessarily be incorrect as it regards with the study and analysis of Physical and Chemical properties of Poisons. Where the Physical properties means the appearance e.g., colour, state, Melting Point, Boiling Point, etc. the Chemical properties has an important characteristic in which the fatal dose, toxicity, action upon the body are studied including its metabolism. The added labour in the field of Toxicology is the detection of any poison inside the body, its Quantitative and Qualitative estimation and interpretation of results of the respective toxicological analysis. Poison may be regarded as any substance which, when taken in sufficient quantity, will cause ill health or death. The adage in this definition is “sufficient quantity”.

Overview

For a Forensic Toxicologist, the mechanism of toxicity often provides perception as to how a chemical or physical agent can cause death or induce incapacitation. If the mechanism of toxicity is understood, descriptive toxicology becomes useful in predicting the toxic effects of related chemicals. Toxicity is the fundamental capacity of a chemical agent to affect an

organism harmfully. These agent are rather termed as Xenobiotics i.e., foreign substance. Xenobiotics include drugs, industrial chemicals, naturally occurring poisons and environmental pollutants. Risk is the possibility of a specific adverse effect to occur. It is often expressed as the percentage of cases in a given population and during a specific time period. A risk estimate can be based upon actual cases or an estimation of future cases, based upon hypothetical calculations. Toxicity rating and toxicity classification can be used for regulatory purposes. Toxicity rating is an arbitrary grading of doses or exposure levels causing toxic effects. These grading may be “super toxic,” “highly toxic,” “moderately toxic” and so on. The most common ratings concern acute toxicity. Toxicity classification concerns the grouping of chemicals into general categories according to their most important toxic effect. Such categories can include allergenic, neurotoxic, and carcinogenic, etc. This classification can be of administrative value as a warning and as information.

Information from the toxicological sciences, gained by experience or research, has a growing influence on our personal lives as well as on human and environmental health across the globe. Knowledge about the toxicological effects of a compound affects consumer products, drugs, manufacturing processes, waste clean-up, regulatory action, civil disputes, and broad policy decisions. The expanding influence of toxicology on social issues is accompanied by the responsibility to be increasingly sensitive to the ethical, legal, and social implications of toxicological research and testing.

Poisoning has become one of the commonest medical emergencies throughout the world because thousands of pharmacological and chemical agents are commonly used and their numbers are increasing every year. The incidences of poisoning and substance abuse have been gradually rising in India over the last few decades. Accurate diagnosis of poisoning is essential, both in the living as well as in the dead, for therapeutic and medicolegal purposes respectively. Poisons are generally detected in body fluids such as urine, blood, or gastric lavage during life, while they are detected in the contents of stomach, bowel and the viscera, besides urine and vomitus, after death.

In a cadaver, one of the better samples to complement blood is vitreous humor, for the reason that it is less likely to degrade quickly, and is equitably easy to collect at time of autopsy. Forensic toxicological analysis has traditionally focused on the use of body fluids including blood along with certain organs in examinations of deaths due to intoxication. However, in

some circumstances, putrefaction and contamination make proper sampling from tissues difficult, such as in exhumation cases. In these cases, bone marrow might be useful as an alternative specimen since it is a potential depot for drugs.

Determination of the toxicological potential of these newly created chemicals became the underpinning of the science of toxicology as it is practiced today. The science of toxicology has expanded to include a wide range of interests, including the evaluation of the risks involved in the use of pharmaceuticals, pesticides, and food additives, as well as studies of occupational poisoning, exposure to environmental pollution, the effects of radiation, and, regretfully, biological and chemical warfare. Nevertheless, it is the forensic toxicologist who has held the title of toxicologist for the longest period of time. The forensic toxicologist is concerned primarily with the detection and estimation of poisons in tissues and body fluids obtained at autopsy or, occasionally, in blood, urine, or gastric material obtained from a living person. Once the analysis is completed, the forensic toxicologist then interprets the results as to the physiological and/or behavioural effects of the poison upon the person from whom the sample was obtained.

All substances can act as poison if they are used in an inappropriate way. The inappropriate way may contain their dosage, state of appearance and mode of administration. For example, normal sugar which is taken in our daily consumption may act as a trigger to diabetic attack if taken in overdose. Similarly, the normal oxygen we inhale may kill a person if even a bubble is injected intravenous. Swiss Alchemist and Physician Paracelsus as well as Italian physician Ramazzini also noted the toxicity of smoke and soot.

Sample types

Table below provides a snapshot of the kinds of samples commonly requested when investigating different manners of death, although there may be unique case needs that have to be addressed for some investigations. For example, exposure to volatile substances requires a sample of the fluid in the lung. Skeletal remains can be useful to determine prior exposure to drugs and other substances. In these cases hair can also be sampled.

Type of death case	*Recommended specimens*
Suicides, motor vehicle crashes, and industrial accidents	Blood, urine, vitreous humour, liver
Homicides and/or suspicious	Blood, urine, vitreous humour, gastric contents, bile, liver, hair
Drug-related	Blood, urine, vitreous humour, gastric contents, bile, liver, hair
Volatile substance abuse	Blood, urine, vitreous humour, lung fluid or tied-off lung, liver
Heavy metal poisoning and exposure to other poisons	Blood, urine, vitreous humour, liver, hair, kidney

Blood and Plasma

Blood is one of the most important physical evidence, which is frequently encountered at the crime scene as a pool of blood, droplet, stains, etc. It can be found in almost every type of criminal activity involving physical violence like murder, assaults, rape, etc at crime scene in form of valuable evidence.

Blood stains are of two types:

1-Visible blood stain

2-Invisible blood stain

Components of blood

The blood, which constitute around 1/13th of the body weight consist of medium plasma and suspended cells like-

1. RBC (Erythrocytes) - Red blood corpuscles containing hemoglobin are responsible for carrying oxygen from the lungs to various parts of the body. They are formed in the bone marrow.

2. WBC (Leukocytes)- White blood corpuscles containing antibodies that fight foreign bodies, which cause infections and disturbs the immune system.

3. Platelets (Thrombocytes)- Platelets are blood cells that help in blood clotting.

4. PlasmaPlasma is the yellowish, liquid portion of the blood that contains electrolytes, nutrients, proteins and vitamins.

Major Functions of blood are

- Transport
- Maintain body temperature
- Control pH (acid-base balance)
- Removal of toxins from the body (Excretion)

Examination of blood

Physical Examination

In the natural light, the blood stains appear as brown, reddish-brown stains, clot or crystals of reddish brown color. If the stain are clear and visible then , examined under UV light (at 230-269nm wavelength.)

Presumptive Screening test

The blood stain obtain from suspected area, contaminated with material should be tested for positive blood stain. Presumptive tests produce a color reaction or release of light, in presence of catalytic property of blood

Phenolphthalein test (Kastel Meyer test)

Phenolphthalein is reduced by Zn powder in a strongly alkaline medium. If this reduced phenolphthalein is oxidized by oxygen liberated by the action of peroxides on hydrogen per oxide (H_2O_2), then a pink or purple color is obtained, if the stain of blood. The sensitivity of phenolphthalein test is about 1:5 lakhs.

Reagent:

Stock Solution

- Phenolphthalein - 2.0 g
- Potassium Hydroxide - 20.0 g
- Distilled Water - 100 ml

- Zinc Dust - 20.0g

Working Solution:

1. Ethanol - 10 ml
2. Phenolphthalein Stock - 2 ml
    a. Distilled Water - 10 ml
    b. Ethanol - 2 ml
3. 3% Hydrogen Peroxide - 10 ml

Reagent Preparation:

The reagent are formed by adding phenolphthalein (2g), potassium hydroxide (20g) distilled water (100ml). Mix, add Zn powder, A few boiling chips and boil under reflux 2-3 hours until the stock solution is formed. Cool and decant into a bottle containing some zinc to keep in the reduced form. Now add ethanol (10ml), phenolphthalein stock solution (2ml), distilled water (10ml), again ethanol (2ml), and 3% hydrogen peroxide (10ml) as working solution. Hydrogen peroxide is used in every colour reaction or in other words, it is responsible for colour obtained in the reaction. If the colour is obtained pink so, it confirms the presence of blood.

Procedure:

A small cutting, swab or extract of the suspected bloodstain is placed on filter paper or spot test paper Two or three drops of ethanol are placed on the stain. Two drops of working phenolphthalein solution are added to the stain. After waiting to insure that no color develops at this stage, two or three drops of 3%Hydrogen peroxide are added. An intense pink color indicates the positive test for peroxides activity and indicates the presence of hemoglobin.

Tetra methyl Benzidine (TMB) Test

Reagent:

Acetate Buffer

- Sodium acetate - 5.0g
- Glacial Acetic Acid -13 ml

- Distilled Water - 57.0.0 ml

Working Solution

- TMB solution - 1.5g
- Acetate Buffer - 20.0 ml

Reagent Preparation

Take 1.5gm of benzidine and 13ml of glacial acetic acid and57ml of distilled water .After shaking benzidine solution is ready for test.

Procedure

Place cutting or swabbing of the stain on filter paper or spot test paper. A drop of TMB Solution is placed on the stain, followed by a drop of 3% Hydrogen Peroxide and mix with glass rod. Appearance of immediate blue-green color is a positive test for peroxides activity i.e indicative of presence of hemoglobin.

Luminol test

- Luminol reagent - Sodium perborate - 0.7g
- 3-Aminophthal hydrazide - 0.1g
- Sodium bicarbonate - 5.0g

Reagent Preparation:

The reagent are formed by taking 0.7g of sodium perborate and 0.1g of 3-aminophthalhydrate is mixed with 0.5g of sodium bicarbonate.

Procedure:

Take the suspected blood stain and add few amount of Luminol reagent appearance of fluorescent color indicate positive test of blood.

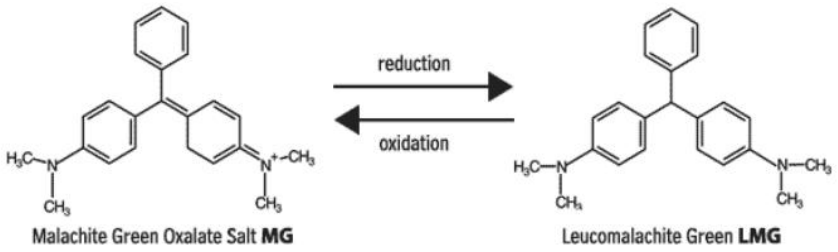
Leucomalachite Green test

Reagent

- Leucomalachite green - 0.1g
- Sodium perborate - 3.2g
- Glacial acetic acid 65%(Solution) - 66ml
- Distilled water - 33ml

Reagent Preparation

The reagent of Leucomalachite green test is formed by the combination of Leucomalachite green(0.1g), sodium perborate (3.2g), and Glacial acetic acid (66ml) in 33ml distilled water. If the color appear green, it confirms the presence of blood.



Procedure

Add Leucomalachite green solution on the stain. If indicate green colour appears it indicate the the presence of blood.

Confirmatory test of blood:

Takayama test - It is also known as Haemochromogen test.

Takayama reagent:

- Saturated solution of glucose - 3ml
- Pyridine solution - 3ml
- 10%NaoH (sodium hydroxide solution) - 3ml
- Glacial Acetic Acid - 7ml

Reagent preparation

Taken the 3ml of saturated solution of glucose, 3ml of pyridine solutiona and 3ml of NaOH solution along with 7ml of Glacial acetic acid.

Procedure:

Place the material to be tested on a microscopic slide and cover with a cover slip. Add a drop of Takayama Reagent and allow to flow under the cover slip. Warm slide gently on a hot plate at 65°C for 10-20 seconds. Allow to cool and observe under microscope at 100X. The appearance of pink needle shaped crystals of pyridine Haemochromogen (Pyridineferroprotoporphyrin) is positive reaction for haeme and confirms the presence of hemoglobin.

Teichmann's test

Teichmann's reagent

KCl, KBr and KI - 0.1 g each Glacial Acetic Acid 100ml. Reagent preparationFor the Teichmann's test, the reagent are formed by the combination KCl, KBr and KI at about 0.1g each in 100ml of Glacial acetic acid. The reagent react with hemoglobin and give brownish rhombic crystal, Confirms the presence of blood.

Procedure

Place material to be tested on a microscopic slide and cover with a cover slip. Let the reagent flow under the cover slip. Warm the slide gently on a hot plate at 65°C for 10-20 seconds. Allow to cool and observe under microscope at 100X. The appearance of brown rhombohedron shaped crystals of ferroprotoporphyrin chloride is a positive reaction for haeme.

Microscopic test

Microscopic Method of examination is having paramount importance relating to identification of blood stain

- Determination of the species of origin by nucleated RBC's along with the cell structure.
- Sex determination by examining chromatin bodies in Leucocytes.
- Detection of blood related pathological condition.

Spectroscopic Method

In this method identification of haemoglobin and its derivatives is done by characteristics absorption band,when viewd through microscope.

Test

A small portion of the suspected stain (as small as 2mm) is put in0.5% Potassium cyanide solution for 15min rest and then fiterate.The filtered thus obtaind is take in 1cm. cell and passed a U-V light in a spectrometer, the absorption observed at from 300 - 600 micron. The absorption maximum at 422 milli micron obtained that the presence of cyclohaemoglobin.

Detection of species origin of blood

The biological evidence has been identified necessarily to determine and confirm whether it is of human origin or not. If it is non-human origin, then to which species it belongs to the species specific proteins in the bloodstains or other body tissues may be identified with the help of species specific antibodies

The species specific proteins from the bloodstain or tissue are extracted in normal saline (8.5 g of sodium chloride in one liter of distilled water) or 5% ammonia solution. The following method are applied to detect the species of origin

Precipitin tube method

Take six precipitin tubes (number can vary on the number of anti sera used) and place them vertically in a precipitin tube stand and label. Put a drop of the bloodstain/tissue extract in the tubes. Carefully add one drop of antiserum for species origin (anti-Human serum, anti- Fowl serum, anti-Dog serum, anti-Cow Serum, anti-Goat serum, etc.) along the walls of tube. Leave undisturbed for 30 minutes at room temperature. Carefully examine the white ring at the interface of two solutions. If ppt is formed, it belongs to that specific anti-serum.

Double diffusion method

In this method both of the reactants, antigen and antibody diffuse towards each other in agar gel plate, and when an antigen combines with its specific antibody at optimum proportions, precipitin are formed. Fill the central well with tissue extract and peripheral wells with

different anti sera for species origin like (anti-Human serum, anti-Fowl serum, anti-Dog serum, anti-Cow Serum, anti-Goat serum, etc.). Cover the Petri dish and keep gel in a moist chamber for overnight. Examine the gel for the presence of precipitin band form.

Urine

Urine is an important biological liquid for forensic analysis. A suspected urine stain may fluoresce pale yellow or pale blue when viewed under long and short wave UV light.

Characteristic odor of urine may be detected by placing a small sample of the stain in a test tube and heating slightly. Urine contains two main constituents, namely, Urea and Creatinine. Both these components are tested for confirming the presence of urine. Urea Nitrate Crystal test & Creatinine Test are employed for such testing.

Urine is also an important exhibit for forensic toxicology analysis. Almost all toxicants or their metabolites can be analysed in urine samples using various analytical procedures.

For identifying Urine	Toxicological Analysis	For individualization
Creatinine test	Color Tests	DNA Analysis
Urea Nitrate test	Chromatographic methods	
	Spectroscopic methods	

Vitreous fluid

Vitreous fluid, or vitreous humor (VH), is a biofluid that is commonly collected and analyzed in forensic toxicology investigations. It is a useful tool for a number of reasons, including:

- Isolated environment: VH is isolated from other body fluids and is preserved from decomposition, bacterial contamination, and other metabolic reactions.
- Reflects premortem conditions: VH reflects the serum concentrations of many elements from the immediate premortem period.

- Estimates time of death: VH can be analyzed for potassium, sodium, and chloride to estimate the time since death.
- Evaluates electrolyte imbalances: VH can be analyzed for electrolytes to evaluate electrolyte imbalances.
- Evaluates hyperglycemia: VH can be analyzed for glucose to evaluate hyperglycemia.
- Tests for drugs and other substances: VH can be tested for certain drugs, viral antibodies, acids, insulin, C-peptide, and some trace metals.

Hair

Hair is common feature for defining characteristics of mammals. The follicle which forms thick end or fine vellus hair eventually covers the human body. The basic process of analysis of hair is depended on hair growth, hair types and care. The human hair is also a vital biomaterial, principally grows as follicle in dermis comprises of protein, particularly keratin. Hair properties depend on hairstyles and hair elimination and vary broadly across different nations and historical eras, as it also used to specify a person's personal beliefs or social position, such as their age, gender, or religion.

The "hair" has two different arrangements:

- The fragment under the skin known as the hair „follicle‟ or when taken from the skin, known to be the bulb. This is situated in the dermis and upholds stem cell that not merely re-grows the hair subsequently after its falls, but also recruited when regrows skin after a wound.
- The shaft is the tough filamentous portion, spreads overhead the skin area. The cross section of the hair shaft generally has roughly three zones.

Every strand of hair is divided into medulla, cortex, and cuticle. The inner region, the medulla, will not always there and is an open, rough region. The second layer of the hair is highly structural and organized cortex which is the actually a source of mechanical support for the hair and helps in water uptake. The cortex comprises filament melanin, which pigment the fibre composed of the numerous, distribution and kinds of melanin granules. The

structure of the follicle analysis the shape of the cortex, that give reference of straight or curly hair. People having straight hair will possess circular hair fibres. Oval and irregularly shaped fibres are commonly more wavy or even curly.

The outer covering is cuticle. Its complicated shape slides as the hair swells and is composed of a single molecular layer of lipid which forms the hair repel water. The diameter of human hair range between 17 to 180 micrometres (0.00067 to 0.00709 in). There are two million small, tubular glands and sweat glands which forms watery fluids that cools the body by evaporation. The glands at the opening of the hair forms a fatty secretion that lubricates the hair and prevents it from dying and lyophobic character.

Hair growth initiates inside the hair follicle that is living part of the hair. The visible portion of the hair is shaft, has no biological activity and is almost considered "dead". The lower portion of the root is known as the bulbs, which comprise the active cells which forms the hair shaft. Other structures of the hair are follicle that contains the oil producing gland. This lubricates the hair and the arrestor pili muscles. This gland gives power to the hair.

Determination of the gender from the hair

The sex of a person cannot be confirmed from hair examination. Length of a complete hair and its refractive index will sometimes be indicative of the sex. Recently success has been observed in determining the sex on the existence or nonexistence of Barr-bodies from the hair root sheath cells.

The absence of Barr body indicates the sex to be of a male while its presence to be that of females.

The males hairs are morphologically much thicker than female's hairs.

Identification of the site of the hair

General features of the hair in determining body areas from where, the hair have been originated.

1. Scalp Hairs: Scalp hairs are extended enough with variable shaft diameter. Medulla lacking to uninterrupted and quite narrow when, associated with its arrangement of hairs from further body areas. These types of hair are with cut or divided ends.

2. Pubic Hairs: Pubic hairs has shaft diameter course, with wide-ranging variation and „buckling".The medulla is relatively broader and mostly unbroken if present. The roots are frequently with follicular tags, and the tips are usually rounded or abraded. They has a stiff texture.

3. Limb Hairs (arm or leg): Limb hairs of diameter are very fine in with slight deviation. The gross presence of hair has arc-like shape. The medulla is broad, discontinuous and with a rough appearance and they have a soft surface.

4. Moustache Hairs: The diameter of the moustache hairs is very coarse with irregular or trilateral cross sectional structure. The medulla is very wide and uninterrupted.

5. Chest Hairs: The shaft diameters of the chest hairs are appropriate and adjustable. The tips are elongated and fine, arc-like and have a stiff texture.

6. Auxiliary or underarm Hairs: They resemble like pubic hairs in overall appearance. The diameter is appropriate and variable, with fewer "buckling" than the pubic hairs. The tips are elongated and fine and have a bleached appearance.

7. Other:

- In case of eyebrow hair: some fluctuation fusiform in appearance.
- In case of eyelash hair: little, stubby with small shaft diameter fluctuation and fusiform in appearance.
- In case of Trunk hair it is a mixture of structures of limb and pubic hairs

Forensic comparison of hair

Hairs, It might be of human or from a wild or trained animal, are frequently observed related with individual hurt accidents and illegal activity, the purpose of the source of hair is time and again concern of detectives and the major means for determination. The microscope evaluation of all kinds of hairs is likely, while head and pubic hairs are best encountered in criminal investigations; others can also be compared with the equivalent techniques.

Types of Forensic Toxicology

Forensic Toxicology is used to help establish cause and effect relationships between exposure to a drug or chemical and the toxic or lethal effects that result from that exposure. Forensic Toxicology talks about to the application of conventional toxicology for the purposes of the Criminal Investigation to assist legal administration. It can be considered as a hybrid of Analytical Chemistry and Fundamental Toxicology with advanced Forensic Medicine. Forensic toxicology is also primarily concerned with the medico - legal characteristics of the detrimental effects of chemicals on human and animals. The expertise of forensic toxicologists is primarily utilized in establishing the cause of death and interpreting its circumstances in post-mortem investigation.

Alcohol

The term 'alcohol' is derived from an Arabic word "Al kohl", which means "something subtle". Alcohol causes a slowing of nerve conduction, which results in slower reaction times, difficulty in processing and assimilating information. The aliphatic alcohol forms a homologous series beginning with methanol, ethanol, n-propanol, isopropanol etc. The first three are readily soluble in water in all proportions but as the carbon chain length increases, water solubility decreases and Octanol is almost insoluble in water. Alcohol beverages are principally a mixture of water and ethyl alcohol with small amounts of other substances, which impart the characteristic odour and flavour to the beverage. These substances are referred as Congeners because they are simultaneously produced during the fermentation process. Congeners consist of organic acids and esters. Alcohol is one of the most useful alcohols for many purposes. Being a central nervous system depressant, it causes an irregularly descending type of depression. Higher centres are depressed first followed by the midbrain and thalamus, spinal cord and finally the medulla. The recovery occurs in a reverse order.

Alcohol is undoubtedly still the largest contributor to impaired driving, but the additional impact of drug impairment on driving has also become a focus in recent years. Forensic toxicologists play a significant role in many aspects of drunken driving, particularly in measuring the pharmacological relationship between drug or alcohol use and impairment. They have a crucial role in analysing samples from motorists suspected of being impaired, interpreting results and presenting material evidence in the court. Ethanol is the most commonly encountered alcohol in terms of drunken driving cases. Consumption of ethanol is

legal in many jurisdictions where it is normally considered as socially acceptable if consumed in moderation.

Other alcohols are occasionally encountered in terms of drunken driving cases. Methanol, is a highly toxic substance and is not generally consumed knowingly but may be consumed by accident, particularly by persons desperate to consume alcohol who are unaware of the toxicity. Methanol is also of major concern as it is frequently been used for adulterating liquors as a cheap substitute of ethanol in the illicit liquor manufacture syndicates, which generally results in fatal cases. Other alcohols such as isopropanol and ethylene glycol can cause impairment but, as for methanol, consumption of these substances is uncommon.

Some Notable Alcohols of Forensic Importance

Ethanol

Ethanol (Ethyl Alcohol) or Grain Alcohol is clear, colourless liquid with typical fruity odour and having burning taste. It is produced mostly by synthetic production from Ethylene. This is mainly done by direct hydration process. Ethanol is soluble in water as well as lipid. The hydroxyl and ethyl groups confer both hydrophilic and lipophilic properties. Thus Ethanol is an “amphophyle”. Its specific gravity is 0.79, which is 1 ml of alcohol weighs 0.79 gm. The ethanol content of various alcoholic beverages is expressed by volume percentage or by proof, the latter being twice the percentage of alcohol by volume. Ethanol is toxic by oral, inhalation, sub-cutaneous, intravenous, intra-arterial, intraperitoneal, and dermal routes. Vaporized ethanol can be rapidly absorbed by inhalation leading to intoxication. Ethanol is a CNS depressant but produces some apparently stimulating effects initially because of depression of inhibitory control mechanisms in the brain. Several antihistaminic, decongestant, multivitamin, and cough syrups contain varying percentage of alcohol from 2 to 25 %. Ethanol has been popular in the past as an antiseptic. Surgical spirit used even today is mostly ethanol with a small quantity of methanol (90 to 95% and 5 to 10 % respectively), along with traces of Castor Oil and Methyl Salicylate.

Methanol

Methyl alcohol is prepared by destructive distillation of wood or molasses. It burns with pale blue non-luminous flame and its vapour produces explosive mixture in presence of oxygen or air. Methanol, also known as Methyl Alcohol or Wood Alcohol, is a chemical compound with

chemical formula CH3OH. It is the simplest alcohol, and is a light, volatile, colourless, tasteless, flammable, poisonous liquid with a very faint odour. It is used as an antifreeze, solvent, fuel and as a denaturant for ethyl alcohol. Because of its poisonous properties, methanol is also used as a denaturant for ethanol. Methanol is rapidly absorbed from the gastrointestinal tract, through lungs and skin. Methanol itself is not toxic but two metabolites formed, formaldehyde and formic acid are highly toxic. These compounds are responsible for causing profound metabolic acidosis and visual defect and blindness.

Isopropanol

Isopropanol or Isopropyl alcohol or 2-propanol is also commonly known as 'Blue heaven' as isopropanol is often highlighted blue to differentiate it from other colourless liquids, which has led to the designation "blue heaven" by the addicts. However, it is a colourless, volatile liquid with a faint odour of acetone and a slightly bitter taste. It is generally used, in massage as Rubbing Alcohol (70%), as a Disinfectant, as Antifreeze, as Paint remover, as cleaning solution, in Toiletries like hair tonics, as after-shave lotion, and as an Industrial solvent. Isopropanol is two to three times more potent than ethanol as a CNS depressant and the usual Fatal Dose of Isopropanol is about 250 to 300 ml. Isopropanol can be absorbed through all routes. In the body it is rapidly metabolised by alcohol dehydrogenase. Approximately 80% is converted to acetone and the residue is excreted unchanged in the urine. Acetone is excreted in the urine and breath, and also metabolised to acetate, formate, and carbon dioxide. Isopropanol may be generated spontaneously in a dead body, presumably due to bacterial or other putrefaction processes. This fact must be borne in mind when subjecting viscera to chemical analysis.

Ethylene Glycol

Ethylene is also known as 1, 2-Ethanediol or Glycol alcohol. It is a colourless, thick, odourless, non-volatile liquid, with a bittersweet taste. Ethylene glycol is not absorbed through skin, and because of its low vapour pressure does not produce toxicity upon inhalation. It is however rapidly absorbed through the Gastro Intestinal tract and is metabolised (more than 80%) to Glycoaldehyde, Glycolic Acid, and Oxalic Acid which inhibit diverse metabolic pathways in the body, including oxidative phosphorylation. Other metabolites include Glyoxylic Acid, Glyoxal, Formic Acid, Glycine, Oxalomalate, Malate,

Benzoic Acid, and Hippuric Acid. The usual fatal dose of Ethylene Glycol is about 70 to 100 ml.

Forensic Examination

Ethanol (Ethyl Alcohol): For the detection of ethanol, following tests are to be carried out in the exhibits.

Iodoform Test: Appropriate amount of the sample is taken and about 1 ml of 5% Sodium Hydroxide solution is added to it and subsequently Iodine solution is added drop-wise with shaking until the liquid becomes persistent dark brown in colour. The whole is left for few minutes. If the Iodine colour disappears, more drops of Iodine solution are added drop wise until persistent brown colour of iodine reappears. Few drops of dilute Sodium Hydroxide solution are added to remove extra Iodine. Equal volume of water is added and left for about ten minutes. Observation of Yellow crystalline precipitate indicates the positive test for the presence of Ethanol.

Dichromate Test: Adequate amount of sample is taken and about 0.2 ml of 2% Potassium Dichromate solution is added followed by about 1 ml of concentration Sulphuric Acid. The yellow colour of the dichromate changes to green or blue indicates the presence of Ethanol.

Sulphomolybdic Test: Sulphomolybdic Acid is actually 1 gm of molybdic acid in 25 ml. of concentrated sulphuric acid. Now for the detection, 2 ml. of the hot reagent is added to 2 ml. of the distillate. A deep blue ring appears at once at the junction of two liquids. On shaking, the whole mixture becomes deep blue. It indicates the presence of Ethyl Alcohol. This test is highly sensitive and is negative with acetone, acetaldehyde and even dilute solution of methyl alcohol. Strong solution of methyl alcohol gives only a light blue colour after several minutes.

Methanol (Methyl Alcohol): For the detection of ethanol, following tests are to be carried out in the exhibits.

Chromotropic Acid Test: Appropriate amount of sample is taken in a test tube and about 2 ml of Potassium Permanganate solution is added to it and shaken well. After that few crystals of Sodium Bisulphate is added with shaking till the disappearance of colour of the solution. About 1 ml of Chromotropic Acid, i.e., 5% of aqueous solution of Sodium salt of Chromotropic Acid and subsequently concentrate Sulphuric Acid is added slowly with inner

sidewall of the test tube to the extent of 15 ml. Appearance of violet colour indicates the presence of Methanol.

Schiff's Reagent Test: About 4.5 ml of sample is taken in a test tube and 0.5 ml of Ethanol is taken. 2 ml of 3% Potassium Permanganate solution and 2ml of Phosphoric Acid is added to it and left for 10 minutes. After that 1 ml of 10% Oxalic Acid is added followed by 1ml of concentrated Sulphuric Acid. The contents are left to cool at room temperature. 5 ml of Schiff's reagent is added further and kept for half an hour. Appearance of purple colour indicates positive test for the presence of Methanol.

Isopropanol (Iso propyl Alcohol): The acid-distillation of the exhibit is used to separate isopropyl alcohol from tissue and other biological material. The distillate is subjected to the following test: 2 ml. of distillate is taken in each of two test tubes and 3 drops of Potassium Permanganate in Phosphoric Acid (by dissolving 3 gms. of $KMnO_4$ and 15 ml. of syrupy Phosphoric Acid in 85 ml. of water) is added in one of the test tubes. This is allowed to stand for 5 minutes. The colour, if any left after oxidation is decolourised with a pinch of Sodium Bisulphite. After that 1 ml of 10% Sodium Hydroxide and 1 ml. of 5% Furfural are added to both the test tubes. The contents of each of the test tubes are filtered into test tubes containing 2 ml. of conc. Hydrochloric Acid. A pink ring is formed at the junction indicates the presence of Isopropanol and the pink colour in the other test tube indicates presence of Acetone.

Postmortem toxicology

Poisoning cases being invariably medicolegal in nature, if the patient dies, an inquest will have to be done, followed by post-mortem examination by a forensic pathologist. This is for the purpose of ascertaining the circumstances in which poisoning may have occurred, and to establish the precise cause and manner of death. The common procedure of examination is the same as for any medicolegal autopsy, with individual attention being paid to those characteristics which can give a clue to the detection of and identification of the poison involved.

The contents of the stomach should be thoroughly examined for traces of poison and also for any distinguishable odour. In case of poisoning, a peculiar smell will be observed on opening the body. The substances detectable by their smell are Alcohol, Cyanide, Carbolic Acid, Petroleum Products, Camphor, Nicotine, Opium, Paraldehyde, Phosphorus Insecticides, and

Pesticides etc. Apart from that, the presence of any foreign material in the form of powder, capsules, tablets, leaves or seeds in the stomach. There will be Laryngeal oedema commonly present in the death due to alcohol and barbiturates. Acute lung congestion and oedema will be detected. Acute swelling of brain with or without a pressure cone may be present on opening of cranial cavity. The urinary bladder will be generally distended. Intravascular sickling may be observed. Most frequently, no signs of trauma or disease in any organs may be visible. Body may decompose faster as compared to the normal conditions of decomposition. The pupils may be dilated or constricted.

Irritation, ulceration and perforation or discoloration and change in colour or softening of the mucous membrane of the stomach. The normal mucous membrane of the stomach is pale and white. Irritant poisons cause patchy redness of the mucous membrane at the cardiac end and greater curvature of the stomach but rarely of the pyloric end. Due to the irritant action of the poison, there may be small haemorrhagic areas along with mucous secretion. Redness of the mucosa of the posterior wall may also be found after death.

Corrosive acids, alkalis and irritants cause softening of greater curvature and cardiac end of stomach as they damage the superficial epithelium. In diseased states such as peptic ulcer or malignancy this softening is uniform and limited to stomach whereas in putrefaction, the softening starts at the dependent parts and involves all the layers of stomach wall and inflammatory signs are absent. Carbolic acid causes hardening and shrinkage of mucous membrane. Ulcers due to corrosives or irritant poisons are present on the greater curvature, have thin, friable margins and surrounded by signs of inflammation. The mucosa is soft and hyperaemic. The perforation of stomach may be found in strong acid such as sulphuric acid poisoning. The stomach is usually black in colour with extensively damaged mucosa. The aperture is large with irregular edge and the coats are lacerated through which acid escapes to the peritoneal cavity causing acute peritonitis.

Chemical Analysis

In every case of death due to poisoning, an attempt must be made to demonstrate the presence of poison by standardised analytical methods. For this purpose, the pathologist conducting the autopsy must collect certain of the viscera and body fluids, and despatch them through the police to the nearest Forensic Science Laboratory. While submitting the samples for analysis it must be ensured that the correct quantity has been preserved in appropriate preservative in

suitable, sealed containers. Since poisons can cause degenerative changes in target organs, histopathological evidence of such damage can be a valuable corroborative adjunct. Microscopic examination of tissues may also sometimes help to substantiate a suspicion of long standing abuse which could have contributed to the cause of death. Tissues submitted for histopathology must always be preserved in formalin. An important proof of poisoning is the detection of poisons in the excreta, blood and viscera. The finding of the poison in the food, medicines act as a corroborative but not a conclusive proof.

The medical practitioner must preserve all the viscera and get it sealed in his presence for onward transmission to the police officer who will forward it to the Forensic science lab for chemical analysis. The viscera along with certain body fluids should be collected, preserved and sent to the Forensic Science laboratory for chemical analysis by the forensic pathologist. The presence of poisons should be demonstrated by standardized analytical methods. The preservative for the viscera is rectified spirit or saturated saline solution. The blood can be preserved in potassium oxalate or sodium fluoride and urine should also be preserved with sodium fluoride.

Sport toxicology

'Doping' is the word used in sport when athletes use prohibited substances or methods to unfairly improve their sporting performance.

The use of doping substances or doping methods to enhance performance is fundamentally wrong and is detrimental to the overall impact of sport. Drug misuse can be harmful to an athlete's health or to other athletes competing in the sport. It severely damages the integrity, image and value of sport, whether or not the motivation to use drugs is to improve performance. To achieve integrity and fairness in sport, a commitment from athletes is critical, but the fans watching their favourite athletes competing also need to demand that athletes succeed. The use of banned performance-enhancing drugs in sport(s) is commonly referred to as doping.

Doping is the intake of drugs i.e. chemical substances and adopting of methods which enhance the performance of sports persons.

To avoid fatigue and to enable the body to reach the utmost limits, the sport persons can use antalgies, cardio-respiratory analeptics, central nervous system stimulants several of which are strong anti-depressants and stimulants.

In sports where body feature or size, whether tall or short are important such as in body building , shape of the body can be modified by hormonal manipulation. Various drugs are used to fight stress, facilitate sleep, and maintain good physical features, such as benzocliapine derivatives and amphetamines cannabinoids alcohol or beta-blockers Methods of doping include blood doping; pharmacological, chemical or physical manipulation, manipulation such as drinking lot of water or taking probenecid before the tests to dilute the effect of the banned substance.

Different Types of Doping Drugs

Narcotics

Narcotics are a type of drug that is used as doping in sports. They are injected into a human's blood stream, or muscles, or under skin. Narcotics can also be swallowed. Illegal, except when prescribed by a license professional. What Narcotics do to a body is they reduce, eliminate, and hide pain. Examples of narcotics are morphine and methadone. Narcotics decrease heart rate, causes nausea, and vomiting. They are a combination compound (ASA [Aspirin] and oxycodone or codeine) are used for moderate inflammation also. Narcotics are can be taken by injected with needles. This drug is used in sports were an athlete does not have a lot of recovery time in between games. Sports like football and hockey are good examples whose athletes commonly use narcotics.

Steroids

The well-known doping drug out there is called anabolic-androgenic steroid. Steroids are a group of powerful compounds that are related chemically to testosterone. Testosterone is the male sex hormones. The original purpose of steroids was to help with different diseases. It was developed in the 1930's. What steroids do to an individual's body is help create more hormones in the body. They can be very useful to people that cannot naturally develop enough hormones for their body. Steroids reduce swelling, pain, and other symptoms of inflammation. The sex hormones for the male are a natural steroid with anabolic effects that can be used medically to build up muscle mass.

Blood doping

Blood doping is a very high intense type of doping. The reason for blood doping is to increase your red blood cell mass and thereby delivering more oxygen to muscle. The procedure in blood doping begins with between one to four units of a person's blood being taken from them. The red blood cells are then separated and stored in a cold area. The blood is then reinfused back into the body about week brier to the athletes' high endurance event

Creatine

Creatine is a lighter form of doping in sports today. It is not as extreme as some of the others doping drugs. Most of creatine out there is not illegal. Creatine is a compound that is made in our bodies. It can be taken as a dietary supplement. This supplement does many things to your body including: provides additional energy for your muscles, volumization of you muscles, Buffer lactic acid build-up, and enhances protein synthesis. It can be consumed by powder, gum, tablets, or liquid. A good sport to take creatine in is explosive sports such as football, and baseball. It is not good to take creatine in a long distance sport. It is not because it can dehydrates you very easily.

Negative effects

Taking Doping drugs can have terrible effects on your body. The well known doping drug, steroids has a number of negative effects to the body. Steroids interrupt the normal development of hormones throughout your body. When this happens your body experiences changes that can not be irreversible. Changes such as sperm production, baldness, breast development in men, breast reduction for women and voice deepens for woman. The negative effects on a persons Cardiovascular System is it increases LDL, and decreases HDL. The risk of high blood pressure is higher.

Also the risk of heart attacks is very high. If a person takes a large amount of steroids it increases irritability and aggression. The doping drug, blood doping, causes much stress on the heart. The reason for this is that your red blood count increases which causes the blood in your blood stream to be thick. The human heart is not used to pumping such thick blood.

Which leads to different kinds of heart diseases. Because this method of doping is taken in by needles (usually shared needles) the person has the risk of AIDS. Narcotics are a street drug. Because this drug is a street drug, many people share needles. This gives the risk of passing different diseases from one person to another.

Overdosing of Narcotics can cause death. Withdrawal effects include limited vision, reduced sex drive, menstrual, chronic constipation, mood swings, and muscle twitches. The doping drug creatine is a fairly new product. The long term effects have not been able to be tested yet. The known effects of creatine is that it can cause cramping, diarrhea, increased urination, and dehydration. If you over dose and take a large amount of creatine the same effects of drinking sea water. This is caused because if creatine sits in your system it can draw water from the body, which causes the intestine to contract.

Analytical methods in Forensic toxicology

Chromatography may be defined as a technique of separating a mixture of components into individual components through equilibrium distribution between two phases. Chromatography is a non- destructive method for determining a multi- constituent mixture of trace, minor, or major constituents into its separate fractions. Chromatography was invented by a Russian Botanist Mikhail Semenovich Tswett in 1906. Chromatography has turn out to be one of the most significant analytical methods for the identification and quantification of compounds in the gaseous or liquid state. The simple principle is based on the concentration equilibrium of the components of concern, between two immiscible phases. One is known as the Stationary Phase, because it is immobilized within a column or fixed upon a support, while the second, called as Mobile Phase, is forced through the first. The phases are selected such that components of the sample have different solubilities in each phase. The differential migration of compounds leads to their separation.

Among all the instrumental analytical techniques this hydrodynamic system is the one with the comprehensive application. Chromatography conquers a dominant position that all laboratories involved in molecular analysis can confirm. Chromatography is a physicochemical method of separation of components within mixtures, liquid or gaseous, in the same manner as distillation, crystallization, or the fractionated extraction. The applications of this technique are therefore several since many of heterogeneous mixtures, or

those in solid form, can be dissolved by a suitable solvent which becomes, certainly, an additional component of the mixture

Significance

Among the immense range of techniques available for biochemical study, chromatography subjugates an honoured position. Hardly any other analytical methods have had such a vital impression on the development of experimental chemistry and toxicology. Applications of this innovative technique to biochemical analysis were readily apparent. Chemical analysis of biological specimens is challenging because they comprise a complex mixture of constituents, many of which react correspondingly with reagents carefully chosen to detect a specific compound. Analytical chemistry is not exclusively intricate when dealing with pure solutions, but the presence of several possibly inquisitive substances, as in biological matrices, creates a challenge. Chromatography resolved that challenge by offering a method to separate components of a mixture, providing a pure specimen that could be measured by any of several analytical methods. At the same time as any technology adapts to the applications for which it is appropriate, chromatography has developed to come across the exceptional requirements of biochemical examination. Capillary gas chromatography modernized the analysis of drugs of abuse, with the capability to resolve congeneric compounds. In the same way, Liquid Chromatography has set way to the greater resolving ability of High Performance Liquid Chromatography (HPLC). High resolution derivatives of electrophoresis, a technique closely allied to chromatography, have made the evolution from research tools into commercial applications. Chromatography endures to advance, and the adaptability of this analytical method appears outstandingly suited to experimental chemistry and toxicology applications.

Liquid Chromatography: Chromatographic separations in Liquid Chromatography, except Size Exclusion Chromatograph, are the consequences of interactions developed between the functional groups of solute molecules, solvent molecules and the stationary phase. The interactions present in Liquid Chromatography are hydrogen bonding, Vander Waals forces and electrostatic forces, and the approaches of Liquid Chromatography are categorised as per the nature of these interactions.

The approach of chromatography is appropriate for a particular separation depends upon molecular mass, polarity and ionic characters of a solute. Different methods of Liquid

Chromatography have developed for scrutinizing the range of mixtures in various kinds of matrices. The methods of Liquid Chromatography include Normal- Phase Liquid Chromatography (NPLC), Reversed Phase Liquid Chromatography (RPLC), Ion-Exchange Liquid Chromatography (IELC) and Size-Exclusion Chromatography (SELC). Selection of a liquid chromatographic approach for a particular analysis also requires selection of a column (stationary phase) and solvents for the mobile phase. Whichever mode is selected for development of a particular method, it is important that certain standards for method validation are fulfilled.

Adsorption Chromatography: The stationary phase is a solid medium to which the species observe through the dual effect of physisorption and chemisorption. The physico-chemical parameter involved here is the adsorption coefficient. It was established first and has a solid stationary phase and a liquid or gaseous mobile phase. Each solute has its own equilibrium between adsorption onto the surface of the solid and solubility in the solvent, the least soluble or finest adsorbed ones migrate more slowly. The outcome is a parting into bands comprehending different solutes. Liquid chromatography by a column comprising silica gel or alumina is an illustration of adsorption chromatography. The solvent that is placed into a column is called the eluent, and the liquid that flows out of the end of the column is called elute. Stationary phases have made considerable improvement since the time of Tswett, who used Calcium Carbonate or Inulin.

Size Exclusion Chromatography: Size Exclusion Chromatography (SEC) is a method by which molecules can be separated according to their size in solution, as a consequence linking indirectly to their molecular masses. To attain this, stationary phases hold pores through which compounds are able to diffuse to a certain amount. Size Exclusion Chromatography is based upon the capability of the sample molecules to penetrate into the highly porous bead- like structure of the stationary phase. Separation occurs only as a result of the different degrees of penetration. Molecules of relatively smaller weight are decelerated in their progression in the column because they can pass into the stationary mobile phase within the pores of the packing.

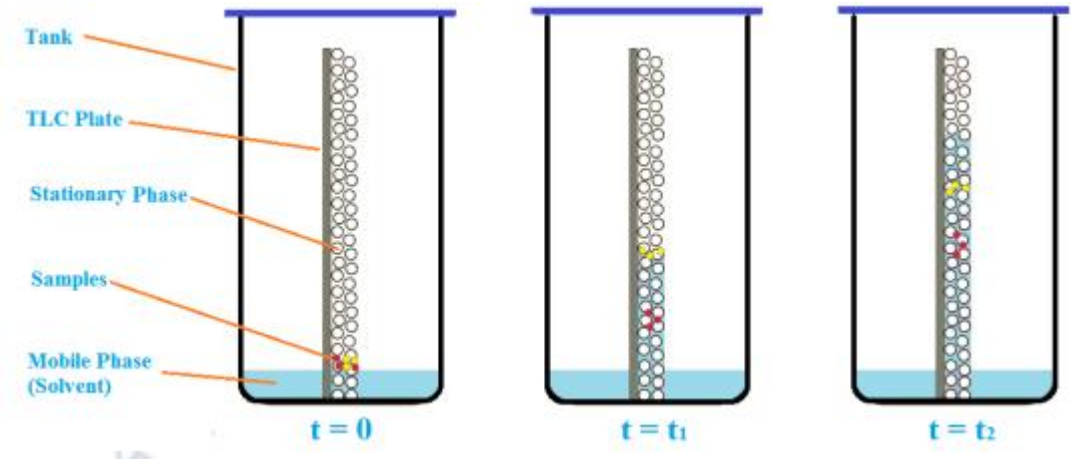
This method is referred to as Gel Filtration Chromatography (GFC) when the stationary phase is hydrophilic and as Gel Permeation Chromatography (GPC) when the stationary phase is hydrophobic.

Partition Chromatography: In this, the immobile phase is a non-volatile liquid that is apprehended as a thin coating on the surface of an inert solid. The mixture to be separated is transported by a gas or a liquid as the mobile phase. The solutes dispense itself among the moving and the motionless parts, with the more solvable constituent in the mobile phase accomplished the end of the chromatography column first. The stationary phase is an immobilized liquid upon an inert and porous material, which has only a mechanical role of support. Impregnation, the oldest procedure for immobilizing a liquid on a porous material, is a technique now out-dated because of the eminent possibility of washing out the column, which is called bleeding.

Supercritical Fluid Chromatography (SFC): SFC works, as its tag proposes, a liquid in the supercritical state as its movable phase. This indicates to developments in the separations of thermolabile compounds and largely for compounds of high molecular weight. The instrument is theoretically a hybrid of gas chromatograph and liquid chromatograph. The instrumentation for Supercritical Fluid Chromatography represents hybrid assemblies of Gas Chromatography and High Performance Liquid Chromatography instrument. To assure the flow rate of the supercritical fluid a syringe pump or a reciprocal pump is used, which is maintained below the critical temperature by means of a cryostat regulated at around 0°C. Gases above their critical pressure and temperature are in a supercritical state, intermediate between that of a gas and liquid. Supercritical fluids have strong extraction properties because the solubility of compounds in fluid is close to that of a true solvent and much lower viscosity permits it to percolate through packed bed of sample. So, not only there is an efficient contact between the extracting fluid and the sample but the fluid is easily removed when it is released from its supercritical state.

Thin Layer Chromatography (TLC): Thin Layer Chromatography (TLC) is an invaluable method used in chemistry and biochemistry. Being economical and sensitive, this technique that is simple to use, can be automated. It has become important essentially since it is possible to carry out several separations in parallel. The principle of the separation between phases of the sample components is though the migration of the constituents through the stationary phase is different. Separation is conducted on a thin layer of stationary phase, usually based upon silica gel and deposited on a rectangular plate made out of glass, plastic or aluminium of a few centimetres in dimensions. To maintain the stationary phase on the support and to assure the cohesion of the particles, an inert binder like gypsum is mixed into

the stationary phase during the manufacture of the plate. The constituents can be identified by simultaneously running standards with the unknown.



High Performance Liquid Chromatography (HPLC): High Performance Liquid Chromatography constitutes a wide-ranging purpose analytical technique derived from the most ancient form of preparative Liquid Chromatography. The contemporary technique is significantly improved in terms of selectivity, resolution, through miniaturization and the use of very elaborate stationary phases. These phases comprise spherical micro-particles with diameters of 2 - 5**µ**m, or porous monolithic material that leads to a significant pressure drop on the column. A large pressure needs to be exerted upon the mobile phase to obtain a continuous flow. This is a type of column chromatography of which a solvent with the chemicals of interest are forced through a thin column at high pressures. The compounds are then separated out based on molecular size and polarity. There are two types of High Performance Liquid Chromatography; one is Normal-Phase High Performance Liquid Chromatography and Reverse-Phase High Performance Liquid Chromatography. The most common type is Reverse-Phase High Performance Liquid Chromatography in which the silica inside the column is actually non-polar. Normal-phase HPLC uses a polar column. High Performance Liquid Chromatography can be used for both quantitative and qualitative information.

High Performance Thin Layer Chromatography (HP-TLC): High Performance Thin Layer Chromatography is an improvement of the technique where the sorbent material (e.g. Silica Gel 60) has a finer particle size and a narrower particle size distribution than conventional Thin Layer Chromatography. HPTLC plates have an improved surface homogeneity and are thinner. The resolution is enhanced, analysis times are shorter and it is sufficient to apply

nanolitres or nanograms of sample (Nano-TLC). After development of the chromatogram to a certain distance, the thin layer chromatography plate is removed and mobile phase is evaporated. The track is scanned in a densitometer with a light beam in visible or ultraviolet range of the spectrum. Depending upon the mode of scanning absorbance or fluorescence is measured by diffuse reflectance.

Breath alcohol test (BrAC)

The Breath Alcohol Concentration (BrAC) test is a non-invasive method used to estimate the amount of alcohol in a person's bloodstream by analyzing their breath. It is commonly employed by law enforcement to determine whether an individual is intoxicated, especially in the context of driving under the influence (DUI).

How the Test Works:

1. *Alcohol Absorption:* After consuming alcohol, it is absorbed into the bloodstream through the stomach and intestines. As blood circulates through the lungs, a portion of the alcohol is transferred from the blood into the air sacs (alveoli) in the lungs.

2. *Exhaled Breath:* When a person exhales, the alcohol vapor from the alveoli is expelled, and the concentration of alcohol in the breath is proportional to the blood alcohol concentration (BAC).

3. *BrAC Measurement:* Breathalyzers are used to measure the alcohol concentration in the breath. They typically use one of three methods:

    - Infrared Spectroscopy: Detects the presence of alcohol molecules by their absorption of infrared light.

    - **Fuel Cell Sensor**: Oxidizes alcohol and produces an electrical current proportional to the amount of alcohol in the breath.

    - **Semiconductor Sensors**: Alcohol vapor interacts with a semiconductor, altering its electrical resistance.

4. **Conversion to BAC**: The BrAC is converted into an estimated **Blood Alcohol Concentration (BAC)** using a partition ratio, which is typically assumed to be 2100:1

(the ratio of alcohol in blood to alcohol in breath). This means that for every 2100 milliliters of breath, it is assumed to contain the same amount of alcohol as 1 milliliter of blood.

Legal Limits:

- In most countries, a BAC of **0.08%** or higher is considered the legal limit for driving, although some countries have lower limits, especially for novice or commercial drivers.

Factors Affecting BrAC Accuracy:

- **Time Since Drinking**: Alcohol absorption varies, and BrAC readings can fluctuate depending on how long it has been since the last drink.
- **Body Temperature**: Elevated body temperature can increase the volatility of alcohol, leading to higher BrAC readings.
- **Breathing Patterns**: Hyperventilation or holding breath can affect the concentration of alcohol in the exhaled breath.
- **Medical Conditions**: Conditions like acid reflux or diabetes can potentially interfere with breathalyzer readings.

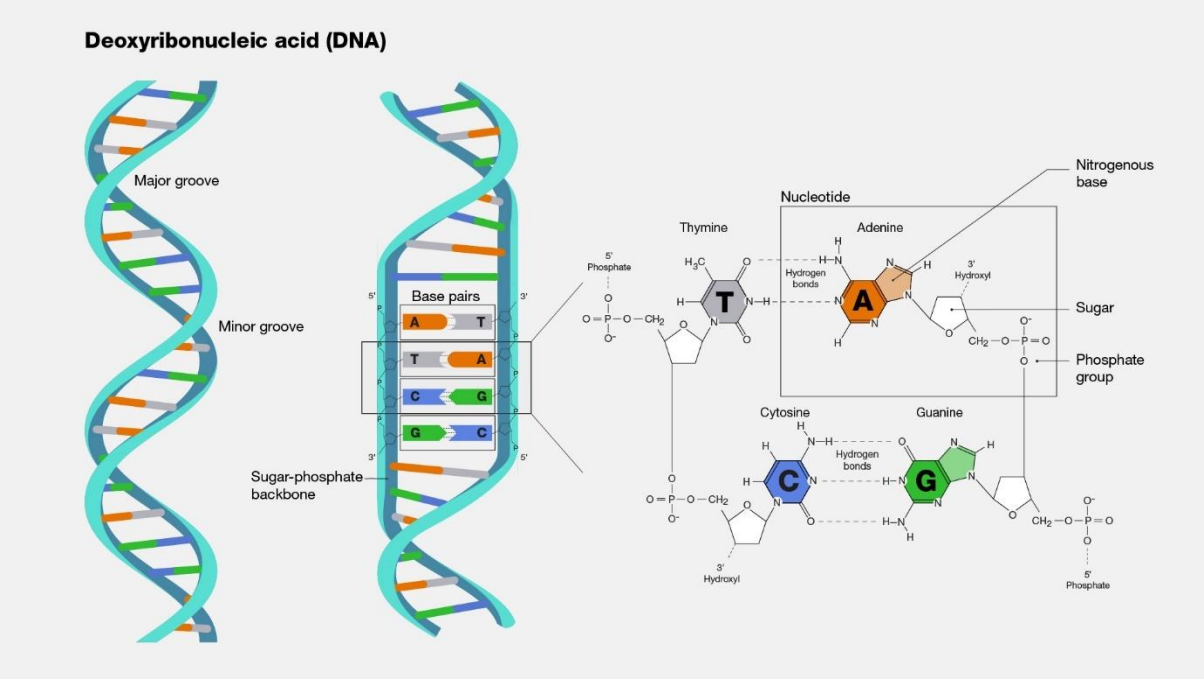
Applications:

- **Law Enforcement**: Used extensively to monitor DUI offenses.
- **Workplace Safety**: Some industries use BrAC tests to ensure employees are not impaired while performing tasks that require full alertness.
- ***Personal Use****:* Portable breathalyzers are available for personal monitoring of alcohol consumption levels.

The BrAC test is favored for its speed and non-invasive nature, but results are generally used as estimates rather than definitive measurements of actual BAC levels.

An introduction to DNA

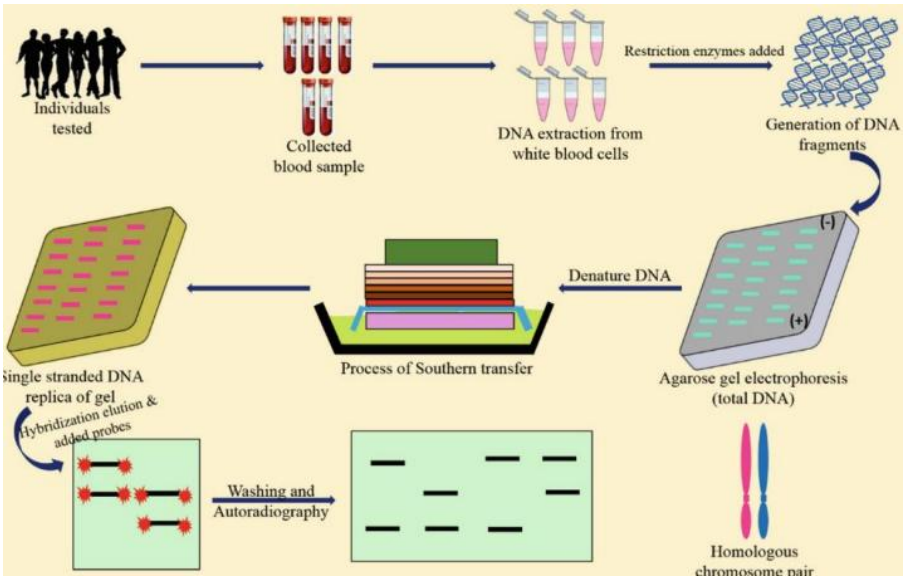
Deoxyribonucleic acid (abbreviated DNA) is the molecule that carries genetic information for the development and functioning of an organism. DNA is made of two linked strands that wind around each other to resemble a twisted ladder — a shape known as a double helix. Each strand has a backbone made of alternating sugar (deoxyribose) and phosphate groups. Attached to each sugar is one of four bases: adenine (A), cytosine (C), guanine (G) or thymine (T). The two strands are connected by chemical bonds between the bases: adenine bonds with thymine, and cytosine bonds with guanine. The sequence of the bases along DNA's backbone encodes biological information, such as the instructions for making a protein or RNA molecule.



Forensic DNA typing

1. DNA profiling is based on Southern Blotting which is used to detect specific genes.
2. 2. The cell sample is taken from the individual by a doctor.
3. The fragments of DNA are extracted from the cells using a centrifuge.
4. Polymerase Chain Reaction (PCR) is used to make many copies of the extracted DNA. DNA is cut into small fragments with restriction enzymes.
5. Later the DNA fragments are separated according to their size by agarose gel electrophoresis and transferred to membrane filter. By doing this, an exact replica of the DNA fragments is made on the agarose gel.

6. The membrane filter of the agarose gel is incubated with a cloned DNA fragment. It is also marked with radioactive label or a stained florescent dye and can be visualized under ultraviolet radiations.
7. The pattern of DNA bands on the autoradiograph helps us to identify a particular gene in the body.
8. A gene probe is used to test a sequence that is highly repeated many times within human genome or minisatellite sequence.
9. Every individual carries a different number of these repeated sequences in the DNA fragment and they lie side by side on the chromosome. They are called Variable Number Tandem Repeats (VNTRs).
10. When genomic DNA is digested using a restriction enzyme, it is then analyzed by Southern blotting. A DNA pattern of their VNTRs is seen.
11. VNTRs are spread over the genome. They are made up of a variable number of end to end duplications of identical and abnormal identical sequences of 2 – 80 base pairs each.
12. Two individuals will not have the same DNA profile except in the case of identical twins



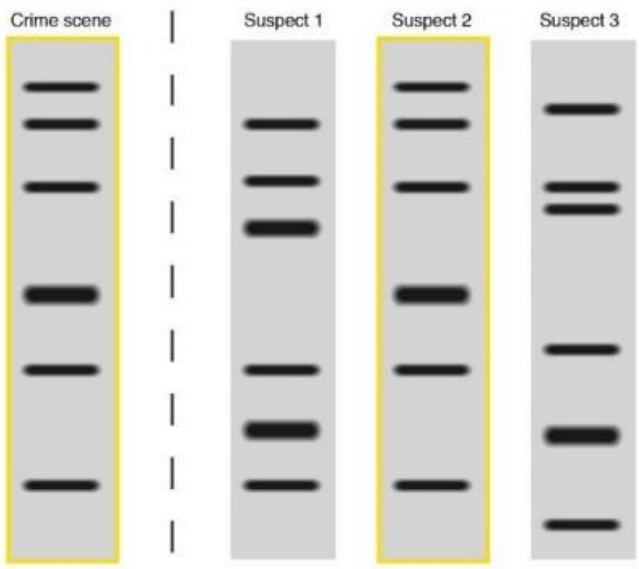


Figure: Detection of Similar DNA Characteristics

Advantages

1. Accurate, affordable and reliable technique
2. Less time required
3. Advanced technology used
4. It can be performed at any age

Limitations

1. DNA samples can easily get contaminated
2. Results need to be interpreted by a professional only
3. Multiple runs needed for each sample
4. Ethical issues may arise such as leaking of individual's person information

Applications

1. To find out genetic defects
2. To identify siblings or twins or paternity or maternity
3. To identify suspects in crime
4. To understand historical migrations

Methods of DNA typing

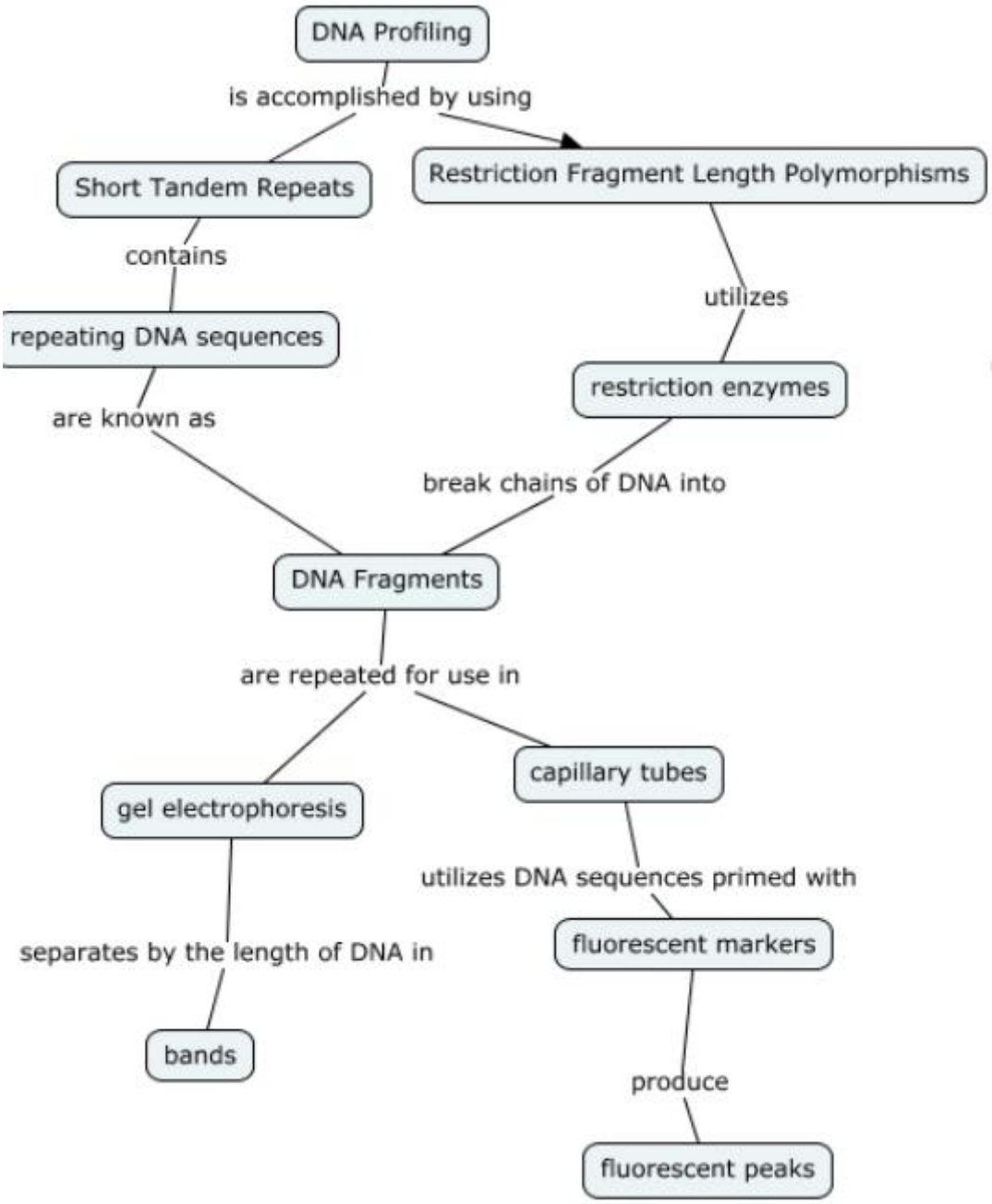


Figure: Methods of DNA Typing

RFLP and PCR methods

Restriction Fragment Length Polymorphism (RFLP) is a technique in which organisms may be differentiated by analysis of patterns derived from cleavage of their DNA. If two organisms differ in the distance between sites of cleavage of a particular restriction

endonuclease, the length of the fragments produced will differ when the DNA is digested with a restriction enzyme. The similarity of the patterns generated can be used to differentiate species (and even strains) from one another. Polymorphisms are inherited differences found among the individuals in more than 1% of normal population.

Applications:

RFLPs can be used in many different settings to accomplish different objectives.

- RFLPs can be used in paternity cases or criminal cases to determine the source of a DNA sample. (i.e. it has forensic applications).
- RFLPs can be used determine the disease status of an individual. (e.g. it can be used in the detection of mutations particularly known muations)
- RFLPs can be used to measure recombination rates which can lead to a genetic map with the distance between RFLP loci measured in centiMorgans.

Restriction Endonucleases:

Restriction endonucleases are enzymes that cleave DNA molecules at specific nucleotide sequences depending on the particular enzyme used. Enzyme recognition sites are usually 4 to 6 base pairs in length. Generally, the shorter the recognition sequence, the greater the number of fragments generated. If molecules differ in nucleotide sequence, fragments of different sizes may be generated. The fragments can be separated by gel electrophoresis. Restriction enzymes are isolated from a wide variety of bacterial genera and are thought to be part of the cell's defenses against invading bacterial viruses. These enzymes are named by using the first letter of the genus, the first two letters of the species, and the order of discovery.

Polymerase Chain Reaction (PCR)

PCR is a technique for amplifying a specific region of DNA, defined by a set of two "primers" at which DNA synthesis is initiated by a thermostable DNA polymerase. Usually, at least a million-fold increase of a specific section of a DNA molecule can be realized and the PCR product can be detected by gel electrophoresis. The regions amplified are usually between 150-3,000 base pairs in length.

PCR/RFLP

Isolation of sufficient DNA for RFLP analysis is time-consuming and labor intensive. However, PCR can be used to amplify very small amounts of DNA, usually in 2-3 hours, to the levels required for RFLP analysis. Therefore, more samples can be analyzed in a shorter time.

Example of use of RFLP in DNA Typing:

1. Extraction: The first step in DNA typing is extraction of the DNA from the sample, be it blood, saliva, semen or some other biological sample

2. Production of Restriction Fragments: The purified DNA is then cut into fragments by RESTRICTION ENZYMES.

3. Electrophoresis: The restriction fragments have negative charge and can be separated by a technique called GEL ELECTROPHORESIS, which separates the pieces of DNA based on their size. The samples of DNA that have been treated with restriction enzymes are placed in separate lanes on a slab of electrophoretic gel across which is placed an electric field. The fragments migrate towards the positive electrode, the smaller fragments moving faster than the larger fragments, thus separating the DNA samples into distinct bands.

4. Detection: The bands can be visualised using luminescent dyes; This approach to DNA typing required quite large samples of biological material in order to obtain reasonable results. For modern forensic work RFLP typing has been superseded by methodology based on the polymerase chain reaction which requires only minute amounts of sample for a successful typing.

Procedures of DNA typing

Here is a step-by-step overview of the DNA typing process:

1. Sample Collection

- *Biological Samples*: DNA is typically obtained from biological sources such as blood, saliva, hair follicles, skin cells, semen, or other tissues.

- *Forensic Samples*: In forensic cases, samples may be collected from crime scenes (e.g., bloodstains, hair, or bodily fluids).

2. DNA Extraction

- *Cell Lysis:* The collected cells are broken open to release DNA. This is done using detergents and enzymes that degrade proteins and membranes while preserving the DNA.

- *Purification:* The DNA is then purified by removing contaminants like proteins, lipids, and other cellular components using techniques like phenol-chloroform extraction or silica-based column methods.

3. DNA Quantification

- Before proceeding, the quantity of DNA is measured to ensure there is enough material for analysis. Techniques such as spectrophotometry or quantitative PCR (qPCR) are used for DNA quantification.

4. Amplification via PCR (Polymerase Chain Reaction)

- *PCR Amplification*: Specific regions of the DNA are amplified using **PCR**. Short Tandem Repeats (STRs) are the most commonly targeted sequences in DNA typing, as they vary greatly between individuals.

- *Thermal Cycling:* PCR involves repeated cycles of heating and cooling to denature the DNA, anneal primers to target regions, and synthesize new DNA strands. This process results in the exponential amplification of the targeted DNA region.

5. Separation of DNA Fragments

- *Electrophoresis*: After PCR, the amplified DNA fragments (especially STRs) are separated using gel electrophoresis or capillary electrophoresis. The DNA is loaded into a gel or capillary tube, and an electric field is applied to separate the fragments by size.

- *Fluorescent Labeling*: In many cases, the PCR primers are labeled with fluorescent dyes to visualize the DNA fragments under UV light or using a laser detector during electrophoresis.

6. Analysis of DNA Profiles

- *STR Pattern Interpretation*: The size of the amplified DNA fragments is compared to known reference samples or databases. The STR patterns at multiple loci (typically 13–20 loci in forensic testing) are analyzed to create a unique DNA profile for each individual.
- *Probability Calculations*: The likelihood of two individuals sharing the same DNA profile is extremely low, especially when multiple STR loci are analyzed. Statistical analysis is used to estimate the probability of a match.

7. Comparison and Matching

- *DNA Database Search*: The generated DNA profile can be compared to known profiles in databases such as CODIS (Combined DNA Index System) in forensic investigations to identify potential matches.
- *Direct Comparison*: In paternity tests or crime scene investigations, DNA profiles from different sources (e.g., suspect vs. crime scene sample) are directly compared for a match.

8. Reporting Results

- *Conclusion:* Based on the DNA profile comparison, a conclusion is reached regarding the match probability or the likelihood of familial relationships.
- *Documentation:* The results are documented in a detailed report that includes the DNA profiles, match probabilities, and an interpretation of the findings.

Types of DNA Typing Techniques:

1. *Short Tandem Repeat (STR) Analysis*: Most commonly used in forensic DNA typing. STRs are regions of non-coding DNA with repeating units of 2–5 base pairs. The number of repeats varies greatly among individuals, making STRs highly discriminating.

2. *Restriction Fragment Length Polymorphism (RFLP):* An older method that involves cutting DNA into fragments using restriction enzymes and separating the fragments by electrophoresis. The pattern of fragment lengths is unique to each individual.

3. *Mitochondrial DNA (mtDNA) Typing*: Used when nuclear DNA is insufficient or degraded (e.g., in old remains). It analyzes mitochondrial DNA, which is inherited maternally and useful for tracing ancestry or familial relationships.

4. *Single Nucleotide Polymorphism (SNP) Analysis*: SNPs are variations at a single nucleotide position in the genome. This technique is useful for analyzing degraded samples and studying population genetics.

Applications of DNA Typing:

- *Forensic Identification:* Matching DNA from crime scenes to suspects or victims.
- *Paternity Testing:* Establishing biological relationships between individuals.
- *Missing Persons Identification:* Identifying unknown remains by comparing DNA profiles with family members.
- *Medical Research and Genealogy*: Studying genetic diseases or tracing ancestry.

This method has revolutionized fields like forensics, enabling highly accurate individual identification and biological relationship analysis.

Unit – 5

Cybercrime Technology and Forensic Science

Use of Computers in Forensic Science

In an era characterized by the ubiquity of digital technologies, the perpetration of cybercrimes has surged, necessitating the evolution of investigative methodologies to match the complexities of the digital landscape. Digital forensics, the process of collecting, analyzing, and preserving electronic evidence, has emerged as a crucial discipline in combating cyber threats. At the heart of this investigative prowess lies the integral role of computers, which serve as both the battleground for cybercrimes and the cornerstone of forensic analysis. As digital footprints proliferate across diverse devices and platforms, the reliance on sophisticated computational techniques becomes imperative for forensic practitioners. This paper explores the pivotal role played by computers in digital forensics, shedding light on their contributions to evidence acquisition, analysis, and the overall investigative process. The symbiotic relationship between technological advancements and forensic methodologies is examined, emphasizing the need for a nuanced understanding of computer systems to navigate the intricate web of digital evidence. As the digital realm continues to evolve, understanding the dynamic interplay between computers and digital forensics becomes paramount in the pursuit of justice and the safeguarding of digital integrity**.**

Role of Computers in Forensic Science

Identification and Preservation

- Imaging: Computers enable the creation of forensic images, which are exact replicas of digital devices. These images allow investigators to analyze the device's contents without altering the original data.
- Data Acquisition: Specialized software tools facilitate the acquisition of data from various sources, including hard drives, mobile devices, and network traffic. This data is crucial for subsequent analysis. Analysis and Presentation
- Data Carving: Computers aid in data carving, a technique for recovering deleted or fragmented files from storage devices. This helps uncover hidden data that may be relevant to the investigation.

- Malware Analysis: Specialized software tools enable investigators to analyze malware samples, identifying their behavior, potential damage, and associated threats.
- Timeline Analysis: Computers facilitate the creation of timelines, which visualize the sequence of events extracted from digital evidence. This helps investigators understand the chronology of the incident.
- Report Generation: Computers support the creation of comprehensive forensic reports that document the investigation findings, including analysis methods, results, and conclusions. These reports serve as evidence in court proceedings.

Additional Roles

- Network Forensics: Computers are essential for network forensics, allowing investigators to capture and analyze network traffic to identify and investigate cyberattacks, data breaches, and other network-related incidents.
- Cloud Forensics: Computers play a crucial role in cloud forensics, enabling investigators to collect, preserve, and analyze data stored in cloud environments.
- Mobile Forensics: Computers are essential for mobile forensics, allowing investigators to extract and analyze data from mobile devices, such as smartphones and tablets

Forensic Databases

Forensic databases are fundamental to the functioning of modern criminal investigations, providing centralized systems for the collection, storage, and comparison of critical forensic evidence that aids in solving crimes, identifying suspects, and exonerating the innocent. These databases store a variety of data types, including DNA profiles, fingerprints, ballistics information, shoeprints, tire tread patterns, toxicology reports, and more. Each type of evidence, when recorded in these systems, can be compared across vast numbers of cases and jurisdictions, helping law enforcement agencies detect patterns and link seemingly unrelated crimes. DNA databases, for instance, allow investigators to match genetic material found at crime scenes with the profiles of known offenders or missing persons, while fingerprint and ballistics databases help trace suspects or link different crime scenes through forensic matches of recovered prints or bullets. Forensic databases are also crucial in identifying unknown remains, supporting missing persons cases, and providing answers in disaster victim identification. Furthermore, they facilitate cross-border crime investigations,

particularly through international cooperation enabled by organizations like INTERPOL, which allows countries to exchange forensic data across their borders, tackling global challenges like terrorism, organized crime, and human trafficking. In forensic science, the accuracy and reliability of evidence comparisons are enhanced through the integration of automated systems, such as CODIS for DNA or IAFIS for fingerprints, which offer rapid, large-scale comparisons in real time. This capability not only accelerates the pace of investigations but also increases the chance of successfully identifying suspects or solving cold cases that may have otherwise remained unresolved for years. As technology advances, forensic databases continue to evolve, expanding their capacity to store vast amounts of data and improving their precision in forensic matching, making them indispensable tools in the pursuit of justice and accountability in both national and international contexts. They ensure that forensic evidence collected at crime scenes can be systematically utilized, bringing science and law enforcement together to enhance investigative outcomes and ensure public safety.

Image Databases

Image databases play a pivotal role in forensic science, serving as critical repositories for various types of visual evidence that can be used in criminal investigations. These databases encompass a wide range of images, including photographs, video footage, sketches, and digital images related to crime scenes, victims, suspects, and evidence. By systematically organizing and cataloging these visual materials, forensic image databases enable law enforcement and forensic experts to efficiently search, retrieve, and analyze critical information that can aid in solving crimes and supporting legal proceedings.

One of the primary uses of image databases is the storage and comparison of photographic evidence collected from crime scenes. Forensic investigators often document crime scenes with high-resolution images to capture detailed information, which can later be analyzed for clues, patterns, or anomalies. These photographs can also be used to create crime scene reconstructions or to illustrate testimony during court proceedings. Additionally, image databases can store mugshots of known offenders, allowing for easy comparisons with images collected from surveillance cameras or other sources, thus aiding in the identification of suspects.

Video footage, such as surveillance camera recordings, is another crucial component of image databases. These videos provide valuable insights into the events surrounding a crime, allowing investigators to analyze the actions of suspects and witnesses. Advanced video analysis techniques can enhance the clarity of footage, improve visibility, and even extract identifiable features, which can then be compared against known profiles in the database.

Moreover, forensic image databases can also include composite sketches created by forensic artists based on witness descriptions of suspects. These sketches can be circulated among law enforcement agencies and the public, increasing the chances of identifying and apprehending suspects. Additionally, databases may store images of evidence collected from crime scenes, such as fingerprints, tool marks, and other trace evidence, which can be analyzed and compared to known samples.

The integration of artificial intelligence (AI) and machine learning technologies is increasingly enhancing the capabilities of image databases in forensic science. These technologies can automate the process of image recognition, pattern matching, and anomaly detection, allowing investigators to quickly sift through large volumes of data to identify relevant evidence. By improving the efficiency and accuracy of image analysis, AI-powered forensic databases are revolutionizing the way visual evidence is utilized in criminal investigations.

Overall, image databases are indispensable tools in forensic science, providing law enforcement agencies and forensic experts with essential resources for gathering, analyzing, and utilizing visual evidence. By enabling quick access to critical information and enhancing the analytical capabilities of investigators, these databases play a vital role in solving crimes, securing convictions, and ultimately ensuring justice in the criminal justice system.

DNA Database

DNA databases are specialized repositories designed to store genetic profiles derived from biological samples, such as blood, saliva, hair, and tissue. These databases play a crucial role in forensic science and law enforcement by facilitating the identification of individuals, linking suspects to crime scenes, and aiding in the investigation of unsolved cases. The power of DNA as a unique identifier stems from the fact that, apart from identical twins, each

individual has a distinct genetic makeup, making DNA evidence highly reliable in criminal investigations.

One of the most prominent DNA databases is the Combined DNA Index System (CODIS), managed by the FBI in the United States. CODIS comprises three levels: the National DNA Index System (NDIS), which contains DNA profiles from convicted offenders, arrestees, and unsolved crime scenes; the State DNA Index Systems (SDIS); and Local DNA Index Systems (LDIS). This tiered structure enables law enforcement agencies to search for matches at local, state, and national levels, significantly enhancing the ability to link different cases and identify suspects.

When biological samples are collected from crime scenes, forensic scientists extract DNA and analyze specific regions of the genome, known as loci, to create a DNA profile. This profile is then entered into the database. When a DNA profile from a suspect or a new crime scene is generated, it can be compared against the profiles stored in the database to find potential matches. A match can provide strong evidence linking a suspect to a crime, exonerate innocent individuals, or identify unknown victims.

DNA databases are also instrumental in the investigation of cold cases, where evidence may remain unexamined for years. Advances in DNA analysis techniques, such as Next-Generation Sequencing (NGS) and Low-Copy Number (LCN) DNA analysis, allow for the analysis of previously collected evidence, which can then be entered into the database for comparison with contemporary profiles. This capability has led to the resolution of numerous long-standing cases, bringing closure to victims' families and delivering justice.

In addition to criminal investigations, DNA databases are also used for non-criminal purposes, such as identifying missing persons and disaster victims. For example, databases can compare DNA profiles of unidentified remains with those of relatives to determine identity, providing critical information in mass disaster situations or when individuals go missing.

While the benefits of DNA databases are significant, they also raise ethical and privacy concerns. Issues surrounding consent, the potential for misuse of genetic data, and the implications of having a genetic profile stored in a database are ongoing discussions within the fields of law, ethics, and public policy. Striking a balance between the utility of DNA

databases in solving crimes and protecting individual privacy rights remains a critical challenge for policymakers and society as a whole.

Overall, DNA databases are invaluable assets in forensic science, enhancing the ability of law enforcement agencies to solve crimes, identify victims, and exonerate the innocent. Their impact on the criminal justice system is profound, offering a scientific approach to addressing legal challenges and ensuring public safety through the accurate identification of individuals based on their unique genetic profiles.

Forensic Archiving of X-Ray Spectra

The development of an X-ray spectral database for forensic science represents a significant advancement in the application of X-ray techniques for criminal investigations and material analysis. X-ray spectroscopy, which includes methods such as X-ray fluorescence (XRF) and X-ray diffraction (XRD), provides valuable information about the elemental and structural composition of materials found at crime scenes. This database aims to compile and organize spectral data that can assist forensic scientists in identifying unknown substances, linking them to potential sources, and supporting legal proceedings.

An X-ray spectral database typically contains spectral profiles generated from various materials commonly encountered in forensic cases, such as metals, plastics, paints, glass, and biological tissues. Each entry in the database includes the spectral data alongside metadata that describes the sample's origin, preparation method, and relevant contextual information. This comprehensive approach ensures that users have access to a robust set of reference materials when analyzing samples collected from crime scenes.

One of the primary motivations for creating such a database is the need for accurate and rapid identification of substances. Traditional forensic methods, while effective, can be time-consuming and may require extensive sample preparation. In contrast, X-ray spectroscopy techniques are often non-destructive, allowing forensic scientists to analyze samples in their original state. This capability is particularly valuable when dealing with limited or precious evidence, as it preserves the integrity of the sample for further analysis or court presentation.

The database can also facilitate the sharing of data across different forensic laboratories and law enforcement agencies, promoting collaboration and standardization in forensic analysis.

By providing a common reference point, the database helps ensure that forensic scientists are interpreting spectral data consistently, leading to more reliable conclusions in criminal investigations. The establishment of such a resource is crucial for enhancing the credibility of forensic science in court, as it allows experts to support their findings with data derived from a wide range of samples.

In addition to aiding in the identification of substances, an X-ray spectral database can also assist in characterizing the physical and chemical properties of materials. For example, the database can be utilized to study the elemental composition of gunshot residue, explosives, or drugs, providing insights into their sources and potential connections to criminal activity. The ability to cross-reference spectral data with known standards can enhance the accuracy of forensic analysis and increase the likelihood of detecting subtle differences that could be pivotal in investigations.

Moreover, advancements in machine learning and data analysis techniques are increasingly being integrated into the development of X-ray spectral databases. These technologies enable the automated classification and identification of materials based on spectral patterns, enhancing the efficiency of forensic investigations. As more data is accumulated and analyzed, machine learning algorithms can refine their predictive capabilities, improving the database's overall accuracy and utility in forensic applications.

Despite the numerous advantages, the development of an X-ray spectral database also presents challenges. Ensuring the quality and consistency of spectral data is paramount, as variations in sample preparation, measurement conditions, and instrument calibration can affect results. Standardization protocols must be established to maintain the integrity of the database and ensure that the information provided is reliable and reproducible.

In conclusion, the development of an X-ray spectral database for forensic science holds immense potential for enhancing the capabilities of forensic investigations. By providing a comprehensive and organized resource for spectral data, the database aids in the identification and characterization of materials, promotes collaboration among forensic laboratories, and ultimately supports the pursuit of justice. As technology continues to advance, the integration of X-ray spectroscopy with emerging analytical techniques promises to further revolutionize forensic science, making it an indispensable tool in the fight against crime.

Video Image Processing and Animation Software

Video image processing and animation software have become invaluable tools in forensic science, significantly enhancing the analysis and presentation of video evidence. These technologies allow forensic experts to scrutinize video footage, extract pertinent information, and create detailed visual representations of events that can be used in criminal investigations and court proceedings.

Video Image Processing in Forensics

1. *Analysis of Surveillance Footage*: Forensic scientists often work with surveillance video to identify suspects, vehicles, and specific actions related to a crime. Video image processing software can enhance the quality of the footage, improve clarity, and reduce noise, making it easier to identify key details. Techniques such as frame-by-frame analysis allow experts to focus on particular moments within the video, which can be crucial for gathering evidence.

2. *Object Tracking*: One of the most significant applications of video image processing in forensics is object tracking. Software can track the movement of individuals or objects across frames, providing a detailed account of their actions during a particular incident. This capability can be instrumental in linking suspects to crime scenes or demonstrating their movements during the commission of a crime.

3. *Facial Recognition*: Advanced video processing tools incorporate facial recognition technology to identify suspects based on facial features. By comparing frames from a video to a database of known individuals, forensic experts can match faces and potentially identify perpetrators or witnesses.

4. *Motion Analysis:* Video image processing can also analyze motion patterns, providing insights into the dynamics of a scene. For example, it can help determine the speed of a vehicle in a hit-and-run case or the trajectory of a projectile in shooting incidents, thus offering valuable data that can aid investigations.

Animation Software in Forensics

1. *Crime Scene Reconstruction:* Animation software allows forensic scientists to create 3D reconstructions of crime scenes based on evidence collected. By integrating data from various sources, such as witness statements, physical evidence, and trajectory analysis, experts can recreate the events leading up to a crime. This visual representation can effectively communicate complex scenarios to jurors, helping them understand the context of the crime.

2. *Demonstration of Evidence:* Animations can illustrate the sequence of events, such as the movement of a suspect or the dynamics of an incident, making it easier for juries to visualize what happened. By visually representing how events unfolded, forensic animators can enhance the clarity of their testimonies and improve the overall impact of the evidence presented in court.

3. *Visualization of Forensic Data*: Animation software can also be used to visualize complex forensic data, such as bullet trajectories, the spread of gunshot residue, or blood spatter patterns. These visualizations can provide jurors and law enforcement with a clearer understanding of how evidence relates to the case.

4. *Educational and Training Tools*: In addition to their use in court, video and animation software can serve as educational tools for law enforcement and forensic professionals. By creating training simulations and visualizations, these tools help enhance the understanding of forensic techniques and evidence analysis.

Challenges and Considerations

While video image processing and animation software offer significant benefits to forensic science, they also present challenges. The reliability of the software and the accuracy of the analysis are paramount; any errors or misinterpretations can lead to incorrect conclusions in a criminal investigation. It is crucial for forensic experts to use validated tools and adhere to established protocols to ensure the integrity of the evidence.

Moreover, the potential for manipulation of digital evidence raises ethical concerns. Courts often require that evidence presented through video processing or animation be subjected to rigorous scrutiny to establish its authenticity and reliability.

Use of Networks in Forensic Science

The integration of networks in forensic science has revolutionized the way evidence is collected, analyzed, and shared across various disciplines. These networks encompass a range of technologies, including data sharing platforms, collaborative databases, communication systems, and advanced analytical tools. By leveraging these networks, forensic scientists can enhance their capabilities, improve collaboration, and increase the efficiency and effectiveness of investigations.

1. Data Sharing and Collaboration

Forensic science often involves multiple disciplines and requires collaboration among various agencies, including law enforcement, legal entities, and forensic laboratories. Networks facilitate data sharing by providing centralized platforms where information can be uploaded, accessed, and analyzed collaboratively. For example, databases that compile DNA profiles, fingerprints, and other forensic evidence enable different jurisdictions to access and cross-reference information, which can be critical in solving cases that span multiple regions.

2. Interagency Communication

Effective communication is vital in forensic investigations, especially when multiple agencies are involved. Networked systems enable seamless communication between law enforcement, forensic experts, and legal personnel. Real-time updates and information sharing allow for quick responses to emerging evidence, which can be crucial during active investigations. This streamlined communication can enhance the overall coordination of efforts, ensuring that all parties are informed and aligned in their investigative strategies.

3. Forensic Databases

The establishment of national and international forensic databases has transformed forensic science. These databases collect and store various types of forensic data, including DNA profiles, ballistic data, and digital fingerprints. For instance, the Combined DNA Index System (CODIS) in the United States allows law enforcement agencies to compare DNA profiles from crime scenes with known profiles to identify potential suspects. Similarly, the Integrated Ballistic Identification System (IBIS) enables the analysis of ballistic evidence to connect firearms to specific incidents. Such networks enable rapid access to critical information that can significantly impact investigations.

4. Remote Analysis and Support

Network technologies enable forensic scientists to conduct remote analyses of evidence, allowing experts to collaborate across geographic boundaries. This capability is particularly useful in specialized fields where expertise may not be available locally. For example, a forensic digital analyst may need to consult a cybersecurity expert to analyze digital evidence from a crime scene. Networked platforms allow for real-time collaboration, ensuring that the best expertise is applied to the case, regardless of location.

5. Integration of Advanced Technologies

Networks facilitate the integration of advanced technologies into forensic science. For instance, machine learning algorithms and artificial intelligence (AI) can be applied to large datasets to identify patterns, predict outcomes, and assist in decision-making. These technologies can enhance the efficiency of evidence analysis, reduce human error, and provide insights that may not be readily apparent through traditional forensic methods. By leveraging networked systems, forensic scientists can harness the power of advanced analytics to improve their investigative capabilities.

6. Digital Forensics and Cybercrime Investigation

With the rise of cybercrime, the use of networks in forensic science has become increasingly critical. Digital forensics involves the recovery, analysis, and presentation of data from electronic devices such as computers, smartphones, and networks. Networked systems enable forensic analysts to conduct remote examinations of digital evidence, recover deleted files, and analyze communication patterns. Additionally, collaborative networks among cybersecurity experts, law enforcement, and forensic analysts can enhance the investigation of cybercrimes by allowing for the rapid sharing of threat intelligence and tactics.

7. Education and Training

Networking technologies also play a role in education and training within forensic science. Online platforms provide access to resources, training modules, and collaborative learning environments for forensic professionals. This facilitates the continuous education of forensic experts, ensuring they stay up-to-date with the latest techniques, technologies, and best practices in the field.

Computer related Crime: Definition and Types

Digital technology is surrounding in all strides of existence, all over the world and has conveyed the factual significance of globalization. At the one end cyber system delivers prospects to communicate and at the other end certain individuals or community exploit its control for criminal purposes. Criminals exploit the Internet and other network communications which are universal in scope. Condition is disturbing that Cyber- crime is an imminent and is talk of the town in every arena of the society and system. Hypothetically and essentially this is an innovative subject for scholars and is developing exponentially. Lot of work has been done and endless has to be done because the innovation or upgradation of novel technology leads to the technical crime, i.e., the digital or the cyber- crime or the ecrime. This is because day by day a new technique is being developed for doing the cybercrime and several times the appropriate investigating technique to challenge that newly cyber- crime is lacking. Cybercrime is any criminal activity involving computers and networks. It can range from deception to unwanted emails also known as spams. It can consist of distant theft of administration or business secrets through unlawful trespass into remote systems around the world. Cybercrime includes anything from downloading proscribed music archives to pilfering millions of moneys from online bank accounts. Cybercrime also comprises non- monetary crimes, such as generating viruses on other computers or posting classified business information on the Internet. Most cybercrimes cannot be placed into a single crime category, which makes statistical recording of this activity limited at best. Internet associated activities are as susceptible to crime and can lead to persecution as effectually as common physical crimes. The forms of crimes that are presently going on have existed long before the Internet was around. By virtue of the gadgets being used today to commit cybercrimes, criminals are now more unspecified and provided with a virtual market of available victims. The accountability falls on individuals to defend themselves and their families through safe online practices. Cyber criminals are no different than conventional criminals in that they want to make their cash as quickly and easily as possible.

Framework for Investigating Computer related Crime

Investigating computer-related crimes requires a systematic approach to ensure thoroughness and adherence to legal standards. The following framework outlines key phases of the investigation process, from initial detection to the final presentation of evidence in court.

1. Preparation and Planning

- *Establish Investigation Goals*: Define the objectives of the investigation, including the scope and specific crimes to be addressed (e.g., hacking, identity theft, online fraud).
- *Assemble a Team*: Form a multidisciplinary team of experts, including digital forensic analysts, cybersecurity specialists, legal advisors, and law enforcement personnel.
- *Develop Protocols*: Create clear protocols for evidence collection, handling, and analysis to maintain the integrity of the investigation and ensure compliance with legal requirements.

2. Detection and Identification

- *Incident Reporting:* Monitor systems for signs of breaches or suspicious activity, and establish channels for reporting incidents from users or employees.
- *Initial Assessment*: Conduct a preliminary analysis to assess the nature and extent of the incident. Identify the type of computer-related crime, involved systems, and potential impact.

3. Evidence Collection

- *Secure the Scene:* Ensure that the affected systems and devices are secured to prevent further damage or tampering. Disconnect from networks if necessary.
- *Data Acquisition:* Use forensic tools to create bit-by-bit images of hard drives, memory, and other storage media. This ensures that original data remains untouched for future analysis.
- *Document the Process:* Maintain detailed records of the evidence collection process, including dates, times, and personnel involved, to establish a chain of custody.

4. Analysis

- *Examine Data:* Analyze the collected data using forensic analysis tools to uncover relevant information related to the crime. This may involve recovering deleted files, analyzing logs, and decrypting data.
- *Identify Patterns:* Look for patterns or connections that may indicate the methods used by the perpetrator, such as IP addresses, timestamps, and network traffic analysis.
- *Evaluate Digital Footprints*: Assess the digital footprints left by the suspect, including social media activity, emails, and online transactions, to gather additional evidence.

5. Reporting

- *Compile Findings*: Create a comprehensive report summarizing the investigation's findings, methodologies used, and evidence collected. Ensure clarity and coherence for the audience (e.g., law enforcement, attorneys, or court).
- *Include Visual Aids*: Use charts, graphs, and visual representations to enhance the understanding of complex data and findings.

6. Presentation of Evidence

- *Prepare for Testimony*: Train team members on how to present findings in court. This includes explaining technical concepts in layman's terms to ensure understanding by jurors and judges.
- *Expert Testimony*: Designate experts to testify about the methods used during the investigation, the integrity of the evidence, and the conclusions drawn from the analysis.

7. Legal Considerations

- *Ensure Compliance*: Throughout the investigation, ensure compliance with relevant laws and regulations, including privacy laws and data protection regulations.

- *Prepare for Legal Challenges*: Be prepared for potential legal challenges regarding the admissibility of digital evidence. Maintain thorough documentation and adherence to best practices to support the validity of the findings.

8. Post-Investigation Review

- *Evaluate the Process:* After the investigation is complete, conduct a review to evaluate the effectiveness of the methodologies used and identify areas for improvement.
- *Implement Improvements*: Incorporate lessons learned into future investigations and update protocols as needed to enhance the efficiency and effectiveness of investigations.

Human Aspects of Computer related Crime

Computer-related crime, often termed cybercrime, has far-reaching implications that extend beyond the technicalities of the crime itself. The human aspects of these crimes involve the motivations behind criminal behavior, the impact on victims, the role of law enforcement, and the broader societal implications. Understanding these elements is crucial for developing effective prevention strategies, policies, and educational programs.

1. Motivations Behind Cybercrime

- *Financial Gain:* Many cybercriminals are driven by the prospect of financial profit, whether through theft, fraud, or selling stolen data. Ransomware attacks, for example, often aim to extort money from individuals or organizations.
- *Ideological Reasons:* Some individuals engage in cybercrime to promote political ideologies or social causes. Hacktivism, a form of cyber activism, involves hacking into systems to protest against perceived injustices.
- *Revenge or Malice*: Personal grievances can motivate individuals to engage in cyber harassment or sabotage. Disgruntled employees, for example, may target their former employers' systems.

- *Thrill-Seeking:* Some individuals are drawn to cybercrime for the excitement or challenge it presents. The anonymity of the internet can make this form of crime appealing to thrill-seekers, especially young individuals.

2. Impact on Victims

- *Emotional Distress:* Victims of cybercrime often experience emotional and psychological distress. Identity theft, for instance, can lead to feelings of violation, anxiety, and paranoia, significantly affecting the victim's quality of life.

- *Financial Loss:* Cybercrimes can result in substantial financial losses for individuals and organizations. Victims may incur costs related to identity recovery, legal fees, or direct theft, creating a lasting financial burden.

- *Loss of Trust:* Cybercrime can erode trust in technology, institutions, and even personal relationships. Victims may become wary of online transactions, hesitant to share personal information, or distrustful of organizations that fail to protect their data.

3. Law Enforcement Challenges

- *Skill Gaps*: Law enforcement agencies often face challenges in addressing computer-related crimes due to a lack of specialized skills and training. The rapid evolution of technology means that officers may struggle to keep up with emerging cyber threats.

- *Jurisdictional Issues:* Cybercrime can transcend geographical boundaries, complicating investigations. Different countries have varying laws and enforcement capabilities, making international cooperation essential but often challenging.

- *Resource Limitations*: Many law enforcement agencies operate under constrained budgets and resources, limiting their ability to investigate and combat cybercrime effectively. This can result in delayed responses and unaddressed cases.

4. Societal Implications

- *Public Awareness and Education:* The rise of computer-related crime has highlighted the need for public education on cybersecurity. Raising awareness about safe online

practices, recognizing phishing attempts, and understanding privacy settings is crucial for empowering individuals to protect themselves.

- *Policy Development:* The prevalence of cybercrime has prompted governments and organizations to develop policies aimed at prevention and response. This includes creating legal frameworks for prosecuting cybercriminals, establishing data protection regulations, and promoting cybersecurity initiatives.

- *Cultural Attitudes:* Societal attitudes towards technology and privacy are evolving in response to cyber crime. Increased awareness of data breaches and privacy violations has led to heightened concerns about how personal information is collected, used, and shared.

5. The Role of Technology

- *Empowerment and Vulnerability*: Technology has empowered individuals by providing access to information and services but also presents vulnerabilities that cyber criminals exploit. Understanding this duality is essential for addressing the human aspects of computer-related crime.

- *Behavioral Patterns*: The use of technology can influence behavior. For example, the anonymity provided by the internet can lead individuals to engage in actions they might not consider in face-to-face interactions, such as cyberbullying or trolling.