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FORENSIC ANALYTICAL CHEMISTRY

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Abstract:

Forensic Analytical Chemistry is defined as analytical chemistry which involves the determination of quantitative or qualitative of unknown samples. Forensic chemists can found at crime scene working with law enforcement professionals or performing analysis on evidence found at crime scenes such as DNA taken from blood stain or saliva. The importance of Forensic sciences is primarily associated with crime and crime scene investigations. This analysis plays major role in criminal justice system

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INTRODUCTION:

Forensic Analytical Chemistry is defined as analytical chemistry which involves the determination of quantitative or qualitative of unknown samples. [1] Forensic science helps to solve criminal case and also assist the investigation to unfold the truth related to a crime.

Forensic chemists can be found at crime scenes working with law enforcement professionals or performing analysis on evidence found at crime scenes such as DNA taken from blood stain or saliva left behind by the suspects while they committing crime.

Nowadays, there are specialists within forensic sciences including criminalistics, DNA profiling or analysis, toxicology and trace evidence among others. Forensic chemistry has wide scope in the field of criminal investigation where it has helped in solving many cases where conventional methods failed to provide any clue about the identity or origin of a suspect.

Forensic science has a rich but undervalued history and a richer potential ahead. Advancements in forensic science are an ongoing process and each day introduces a new technique into the forensic world.

At present forensic investigators has the support of numerous tools as well as medical and scientific support in their field of work. The importance of forensic science is primarily associated with crime and crime scene investigations. Forensic science is one of the most promising and rewarding careers today. It has a wide range of fields including anthropology, forensic law enforcement, medical, biological and many more.

Forensic DNA analysis or DNA profiling was first introduced in 1981 and since then it has been established into a powerful tool for practicing criminal justice to crack the cases on crime scenes. Forensic sciences focus on the use of genetic material in criminal justice system to answer questionsrelated to legal concerns including civil and criminal cases. What makes DNA so useful and powerful is the uniqueness of DNA. While each person has 99.9% of human DNA sequences need only 0.1%of DNA's unique sequences. This analysis plays a major role in criminal justice system. [3] Forensic identification is the universal method used to establish the veracity in the process of forensic investigation. Both criminalities and medico-legal identification are integrative parts of forensic identification. The value of an identification method resides in the specialist's ability to compare traces left at the crime scenes with traces found on other materials such as reference evidence. Through this procedure,one can compare traces of blood, saliva or any biological sample left at the crime scene with those found on a suspect's clothes and with samples from the victims.

Medico-legal identification is based on scientific methods or intrinsic scientific methods absorbed from other sciences. Scientific progress in the last 30 to 40 years has highlight the role of the specialists in identifications.

Their role proves its significance in case that have to do with civil, family, and criminal law, as well as in cases of numerous victims (accidents, natural disasters, terrorist attacks and wars). In field of forensic genetics Polymerase chain reaction (PSR) is used by studying a set of DNA fragments that proved to have unique characteristics in some individuals which were intrinsic and non-recurring. The only exception for this is identical twins.

Forensic analysis is comparative in nature. Two classes of analysis are recognized at the forensic laboratory (1) Screening, (2) Legal analyses. In screening, speed and the order of magnitude of concentration are of the essence. The desired data are to be used for forensic treatment or investigation of the source; thus, speed if of the utmost importance. The first category of samples becomes the second as soon as as the treatment begins. All positive samples are handled as legal samples if a positive result is obtained. The second category applies to legal cases, in which qualitative and quantitative information, as well as sample integrity are necessary. Legal cases should be identified as such from the outset in order to preserve this distinction. One of the powerful applications of electro analytical chemistry in forensic sciences is capillary electrophoresis.

History of forensic analytical chemistry:

During the early 19th century there was no accurate method to determine the amount of chemicals present in a particular substance and the people who commit the crimes related to poisons where not punished appropriately.

Throughout the history a lot of poisons were used to commit murder which may include arsenic, nightshade, hemlock, strychnine and curare.

- In 1832, the first major contribution to forensic chemistry was introduced by British chemist James Marsh. He created Marsh test for arsenic detection, which was used for investigation of murder trial[4]. During this period forensic toxicology gained a lot of attention.
- Mathieu Joseph Bonaventure Orfila, the father of toxicology has made great advancements during this century. He helped to develop tests for the presence of blood in a forensic context and is created as one of the first people to use microscope to assess blood stain
- Alphonse Bertillon of France is known for the development of the first scientific system of personal identification. In 1879, he developed the science of Anthropometry which is a systematic procedure of taking a series of measurements to distinguish one individual from another.
- Francis Galton of the U.K did the first systemic study of fingerprints. He developed a methodology for classifying fingerprints for filling purpose. In 1892, he published a book named “fingerprints” which had good statistical proof stating the uniqueness of fingerprints in personal identification.
- Hans Gross of Austria was a lawyer by profession. He dedicated many years to developing the principles of the criminal investigation.
- Jean Servias Stas, a chemistry professor from Belgium was the first to successfully identify vegetable poison in body tissue.
- Dr Edmond Locard was a French criminalist who was also known as “Sherlock Holmes of France” was a pioneer in forensic science and criminology. He established the first police crime laboratory[5].
- In 1960, Lucas described the application of gas chromatography to the identification of petroleum products in the forensic laboratory.

Theory:

Forensic chemistry is a branch of forensic sciences that involves the study of physical, chemical and biological evidence of a crime. Forensic chemistry within chemical science that uses chemical and toxicological application to identify unknown variables in crimes scene and finding important clues using chemistry[6]. The tests are carried by specialists known as forensic chemists who analyses and explains the evidence and testimony collected from the crime scenes. Then they attempt to identify and connect the pieces together. This is done by using chemical tests on the collected materials[7]. Forensic analysis is the application of analysis to law enforcement. Analytical chemistry is the science that addresses methods used to determine the

quantitative or qualitative composition of unknown samples.

Forensic toxicology:
The term `forensic toxicology' covers any application of the science and the study of poisonsto the elucidation of questions that occur in judicial proceedings. The subject is usually associated with work for the police, the corner and the criminal law courts. However, the analysis and identification of medicine and the maintenance of agriculture, industrial and public health legislationare all aspects of forensic toxicology.

The forensic toxicologist is expected to detect and identity poisons. Chemical analysis is used to detect the presence of poison, measure its concentration and relate this to its known toxicity. Forensic toxicology demands overall analytical systems designed to exclude or indicate the presence of any poison in each chemical groups.

For example, Trestrail estimated that out of 679 documented homicidal poisonings, 31% resulted from arsenic,9% from cyanide, with only 6% from strychnine and 2% from morphine.
Orifila was well acquainted with this aspect of forensic toxicology his principle as follows:

- All chemists who undertake this work must have toxicological experience.
- The analyst must be given a complete case history that contains all the information's available.
- All the evidential material, suitably labelled and sealed in clean containers, must be submittedand examined.
- All the known identification tests should be applied and adequate notes made at time.
- All the necessary reagents use for these tests should be performed to establish this fact.
- All test should be repeated, and compared with control samples to which the indicated poison has been added

Types of forensic analysis techniques:
Forensic analysis carried out by the two types of techniques:

- Organic analytical techniques
- Inorganic analytical techniques

Organic analytical chemistry:
The organic analytical techniques are carried out for the analysis of organic compound s suchas alcohols, aldehydes, alkenes, carboxylic acids, phenols, ketones etc. in the unknown substances. The analysis provides the identity (qualitative result) and the amount (quantitative result). Various analytical techniques used for analysis of organic compounds are

a) Chromatography
b) UV- visible spectrophotometry
c) Infrared spectrophotometry
d) Mass spectrophotometry
e) Gas chromatography-Mass spectroscopy
f) Liquid chromatography-Mass spectroscopy

Inorganic Analytical Chemistry:
The inorganic analytical technique is carried out for the analysis of inorganic elements suchas C, B, Na, Mg, AL, FE, S, AG, Pb, CU etc. in the unknown substances.

Various analytical techniques used for analysis of organic compounds are

a) Atomic absorption spectrophotometry
b) Neuron activation analysis
c) X-ray diffraction analysis
d) Fluorimetry
e) Nephloturbididty
f) Atomic emission spectroscopy

Analytical requirements:
The forensic toxicologist should remember Orfila's maxim 'The presence of poison must be proved in the blood and organs before it can be considered as a cause of death'. There are four stages in any toxicological examinations:

Detection:
To detect any drugs or poisons in the samples submitted by means of screening procedures.

Identification:
To identify conclusively any drugs, metabolites or poison presents.

Quantification:
To quantify accurately those drugs, metabolites or poison presents.

Interpretation:
To interpret the analytical findings in detection and quantifications in the context of the case, the information given and the questions asked by the investigating officer.

Collection And Handling of Material at Crime ScenesMedicaments and other materials:
Empty or nearly empty, tablet containers found near the body gives the first clue that the person may tried to commit suicide. But it mistakes to spend too much time identifying every tablet or capsule found at the spot. However, a quick attempt the identification before analyzing body fluids can be valuable for the identification of the solid dosage forms.

Food or drink residues:
Stomach contents should be examined visually for the color and presence of tablet residues or excipients. The odour should be noted as this may indicate the presence of alcohol, aldehyde, ketones, phenols, cyanide, nicotine etc. Sample of food particles which is found in the buccal cavity gives a good evidence and evaluations for the forensic studies. Some food does have natural toxins which can be formed in food as defense mechanism of plants.

Examination of GIT contents is a critically important investigation in forensic autopsies to establish the condition, and also provides valuable clues in the elucidation of accidents, homicides, suicides etc...

The priority given to this aspect depends on the case history, but the rapid initial examination at an early stage is often useful.

Vegetable poison:
To test for pesticides, users simply pass a swab over the fruit or vegetable, insert the swab intothe detector and wait about 30 seconds. A green light on the face of device means the pesticide residue is under the EPA tolerance; a red light means the opposite.

Blood:
- Whole blood is considered as one of the widely used sources of DNA. It is preserved in an anticoagulant (Ethylenediamine tetra acetic acid) and conserved at 4^0C for 5-7 days initially.
- After this period, DNA samples are kept at -20^0C for few weeks or at -80^0C for longer periods of time.
- Epithelial cells collected from crime scenes are harvested with sterile brush or bud.
- After harvesting, they are wrapped in plastic envelope and kept in a dry environment at room temperature.
- It is essential that proper care is taken, such as maintaining integrity of the crime scene, wearing face masks and full protective suits during the investigation of scene, as inappropriatehandling of the evidence can lead to serious consequences.
- In worst cases, cross-contamination leads to high level of sample degradation; this can confuse or avert the final result of evidence.

Hair specimen:
Hair samples are primarily collected using tweezers. Fibers are threadlike elements from fabric or other material such as carpet most are easily identifiable under a microscope.

To collect pulled hairs, be sure to wear new, unused, clean latex or nitrile gloves and pull 25 hairs (or have the suspect or person of interest do it themselves) from various areas of the scalp or pubic region. Place these in an evidence or pharmacy fold, place the fold in an envelope and properlylabeled.

The most common methods used to collect hair and fiber evidence include the following:
- Visual collection:

On some surfaces, hairs and fibers can be seen with the naked eye
- Tape lifting:
- Vacuuming.

Hair analysis can be done in three ways:
- Testing the hair shaft for drugs or nutritional deficiency in aperson's system.
- Analyzing DNA collectedfr

Analytical procedure for the examination of food and drinks:

- Most poisoning cases and many extortion attempts, involve the addition of toxic substance tofood or drink.
- The analysis of suspected poisoned food or drink presence different problems from the detection of the drugs and poisons in body fluids.
- The concentration of the toxic agent is usually higher than that found in blood, urine or post mortem tissue samples, but the available material for analysis is often more limited, and of much greater variety.
- The routine analysis of blood and urine samples soon ensure recognition of endogenous substance (example; Urea, cholesterol, etc.) and of common exogenous chemicals (eg. Caffeine and nicotine) when they occur in extracts, spectra and chromatogram.
- Packet labels demonstrate that even simple food stuffs may contain several unfamiliar chemical additives. The parallel analysis of a purchased sample of the same food may be necessary to check that unavoidable reactions or unfamiliar UV spectra are normal or abnormal for that type of material.
- Depending on the case history, it is sometimes useful to request a urine and a blood sample from the victims; they may have been poisoned, but not by the food or drink submitted for analysis.
- Alternatively, the food may contain a poison, but there may be no evidence of the poison in their blood or urine. Fake poisoning is not uncommon, especially in marital cases.
- Another type of fake poisoning is the misguided joke of putting some obnoxious materials (e.g. Urine, aloes, soap, mustard) into a person's food or drink.
- The method described below is designed to obtain the maximum amount of analytical data from the minimum quantity of the material.
- It is very flexible and can be applied to tea, coffee, milk, alcoholic drinks, sandwiches, cakesand other numerous types of food. Vomit and food stains may also be examined.

Procedure:

- Make a full description of the exhibit, noting the type of the container, labels, seals, stains or identification marks on the outer surface and total volume or mass of the contents.
- Note the colour, odour, possible presence of injection marks, presence or absence of the suspended solids or sediments, pH and whether material has a tendency to form a stable frothon shaking.

Colour:

The appearance of abnormal colour may indicate the presence of inorganic pigments (e.g. Coper, nickel or cobalt salts), dyestuffs from the tablets, capsules, medicine, pesticides and rodent baits, especially those that contain warfarin, reserpine, chloralose or diphenadione. Common rat-bait colours are blue, green or red, and are usually associated with oatmeal or cereal grain.

Odour:

Odour is often very characteristic, and therefore smelling an exhibit can be very revealing, there is always the inherent danger of inhaling toxic vapours and this should be borne in mind when conducting such tests. Material taken straight from the refrigerator should be warmed gently prior to examination for odour. If possible, the opinions of several colleagues should be obtained on any abnormal odour. Many poisons can be detected in this way, but the test is very subjective, and some people have a poor sense of smell (eg. Cyanide detection by odour is an inherited ability). In favourable circumstances it is a very sensitive test; for instance, cyanide, chloroform and toluene canbe detected for about 1 ppm.

Sediments and suspensions:

Sediments mat result from insoluble or sparingly soluble tablets or capsule excipients (eg. Talc, starch or calcium phosphate), certain poisons (eg. Arsenious oxide) or sometimes from an interaction of the added contaminant with the beverage or drink (eg. Battery acids produces coagulation of protein materials).

The presence of insoluble inorganic crystalline is often caused by the addition of cleaning powders. When crystalline material can be isolated from the

materials under examination, X-ray diffraction and infra-red (IR) spectrophotometry are valuable nondestructive techniques.

Frothing:
The presence of a stable froth when the sample is shaken may indicate contamination with soap or detergents. Acidify the sample, shake again and, if froth still forms a detergent rather than soap is present. Although of low toxicity, soap, detergent and cleaning powders are frequently the cause of compliance of poisoning or adulteration.

Direct ultraviolet spectrophotometry:
In poisoned food or drink sample, concentrations of 0.1 to 10% of toxic agents are not uncommon, so that direct UV spectrophotometry is often used. If a sample of food content contains a foreign substance with an A_1^1>200 and it is present at a concentration of 0.1%, the material may be diluted 50 times and still show the characteristic spectrum for the substance when examined by direct uv spectrophotometry. This is very useful, rapid and non-destructive exclusion method for the large number of drugs, disinfectants and pesticides with high specific absorbances. Direct uv spectrophotometry is invaluable asan initial screening technique for all alcoholic drinks, and for beverages such as tea, coffee, milk and soup. It may also be applied to solid foods (cakes, sweets etc.) and to complete meals (meats, stews, vegetables), either by application to a diluted homogenate or by examination of copious water-wash of the food material. The later method is preferred, as most poisonous tend to sprinkle the poison on the surface of the food rather than add it during the cooking process. If possible, a control sample of the particular food or drink should be examined at the same time. If the spectra obtained with the control sample are similar to those obtained with the sample, a large number of substances are excluded without loss of destruction of what may be an extremely limited amount of original material.

Colour tests:
After uv spectrophotometry, the diluted liquid or washing is available for further preliminary tests, using a small amount for each test:

- Apply the Fujiwara test for trichloro-compounds. The aqueous layer in this test can be usedto test for the presence of oxalate.
- Apply the diphenylamine test for oxidizing agent.
- Test for the presence of halides with silver nitrates.

Analytical procedure for examination of tablets and capsules:
Most tablets and capsules are complex mixtures that contain a small amount of one or more drugs together with a larger number of excipients. A combination of visual and microscopic examination, colour tests, chromatography and UV and IR spectrophotometry is employed in the analysis. The first step is to make careful notes about the container and its contents. The container may be a tube, envelope, folded paper packet, box or bottle. Note any seals and labels. Make full note on information given on the label. For the contents note the total number of tablets or other preparations in the container, together with an estimate of total number that the container could hold if full. Note the shape, whether compression coated, film-coated, enteric-coated or sugar coated, exterior and interior colour, layered and core tablets, mean mass, diameter and thickness.

Procedure:
The presence of plant material indicates the tablet is probably a herbal remedy. These are frequently very complex mixture and are unlikely to be identified by simple methods. However, examination of an alcohol extract by UV spectrophotometry and TLC is worth attempting.

Odour:
A few drugs have distinctive odours, which can provide a clue to the compound or at least to thetype of preparation. A yeast-like or meaty smell may indicate harmless vitamin preparation; an odour of the peppermint may indicate a non-toxic indigestion remedy. Antibiotics, especially of the penicillin group, sometimes have a rather unpleasant sulphide-type smell. Other drugs with characteristic odours are phenelzine, chloralhydrate etc.

Size:
The size of the tablet, which should be measured

with callipers having a vernier reading to 0.1mm, can provide a rough guide to the general class of preparation. Tablets of large diameter (i.e., >10mm) are unlikely to contain potent alkaloids (except codeine) and, conversely, tablets with diameters less than 5mm could not contain drugs with medicinal doses of 200mg or more.

Forensic DNA Analysis:
DNA was first described by Watson and Crick in 1953, as double stranded molecules that adopts a helical arrangement. Each individual's genome contains a large amount of DNA that is a potential target for DNA profiling.

DNA is often described as the ''blue print of life' 'because it contains all the information that an organism requires in function and reproduction. The model of the double-helix structure of DNA was proposed by Watson and Crick. The DNA molecule is a polymer of nucleotides. Each nucleotide is composed of a nitrogenous base, a five-carbon sugar(deoxyribose), and a phosphate group. There are four nitrogenous bases in DNA, two purines (adenine and guanine) and two pyrimidines (cytosineand thymine). Each base is attracted to its complimentary base: adenine base always pairs with thymine base whereas cytosine base always pairs with guanine base. There are two complete copies of genome in each nucleated human cell. Human contains ~3,200,000,000 base pairs of information, organized in 23 pairs of chromosomes. There are 2 sets of chromosomes; 1 version of each chromosome is inherited from each parent with total 46 chromosomes. Based on the structure and function, classification of Human genome into following different types:

Coding and regulatory regions:
The regions of DNA that encode and regulate protein synthesis are called genes. Approximately, a human genome contains 20,000 to 25,000 genes; 1.5% of the genome is involved in encoding for proteins.

Noncoding:
Overall, 23.5% of the genome is classified under genetic sequence but does not involve in enclosing for proteins; they are mainly involved with the regulation of genes including enhancers, promoters, repressors and polyadenylation signals.

Extragenic DNA:
Approximately 75% of the genome is extragenic, of which 50% is composed of repetitive DNA and 45% of interspersed repeats. Four common types of interspersed repetitive elements are:

(a) Short interspersed elements
(b) long interspersed elements
(c) long terminal repeats
(d) DNA transposons.

Tandem repeats consist of three different types:

i. Satellite DNA
ii. Minisatellite DNA
iii. Microsatellite DNA

Forensic Genetic:
DNA loci that are to be used for forensic genetics should have the following ideal properties:

- Should be highly polymorphic.
- Should be easy and cheap to characterize
- Should be simple to interpret and easy to compare between laboratories.
- Should have a low mutation rate.

Biological material:
Three most important steps are:

- collection
- characterization
- storage.

Sources of Biological Evidence:
Human Body is composed of trillions of cells and most of them are nucleated cells, except forthe blood cells. Each nucleated cell contains two copies of individual's genome and can be used to generate a DNA profile. Usually, samples show some level of degradation but when the level of degradation is high, more cellular material is needed to produce a DNA profile. Biological samples with nucleated cells are essential for forensic genetic profiling, such as:

- Liquid blood or dry deposits.
- Liquid saliva, semen, or dry deposits.
- Hard tissues like bone and teeth
- Hair with follicles

Characterization of DNA Analysis:
Analysis of DNA involves four basic steps, which are as follows:

- DNA Extraction
- DNA Quantification
- DNA Amplification
- Detection of the DNA-amplified products

DNA Extraction:
The first DNA extraction was performed by Friedrich Miescher in 1869. Since then, scientists have made progress in designing various extraction methods that are easier, cost-effective, reliable, faster to perform and producing a higher yield.

With the advent of gene editing and personalized medicine, there has been an increase in demand for reliable and efficient DNA isolation methods that can yield adequate quantities of high quality adequate quantities of high quality DNA with minimal impurities.

There are various methods of extraction as mentioned below, though commonly used are Chelex-100 method, silica-based DNA extraction and phenol-chloroform method.

1. Chromatography-based DNA extraction method.
2. Ethidium bromide-cesium chloride(EtBr-CsCl) gradient centrifugationmethod.
3. Alkaline extraction method.
4. Silica column-based DNA extraction method.
5. Salting-out method.
6. Cetyltrimethylammonium.
7. Phenol-chloroform method.
8. Sodium dodecyl sulfate (SDS)-proteinase K method.
9. Silica column-based paper method.
10. Magnetic beads method.

Cellulose-based paper methodChromatography-Based DNA Extraction method:
Chromatography-based DNA extraction method is used to isolate DNA from any kind of biological material.
This method is divided into three different types:

Size-Exclusion chromatography:
In this method, molecules are separated according to their molecular sizes and shape.
In this method samples are allowed to the column and the separation are take place based onthe shapes and size

Ion-exchange chromatography (IEC):
In this method, solution containing DNA anion-exchange resin selectively binds to DNA with its positively charged diethylaminomethyl cellulose group. This method is simple to perform when compared with other DNA extraction methods.

Affinity chromatography:
Protocol is similar to IEC; however, it uses oligo that forms specific interaction with nucleic acid resulting in separation from the cell lysate.

- This procedure is used for isolation of messenger ribonucleic acid(m-RNA).
- It is time-efficient
- It yields as very good quality of nucleic acid.

EtBr-CsCl Gradient Centrifugation Method:
In 1957, Meselson developed this method.
Procedure:

- DNA is mixed with CsCl solution, which is then ultracentrifuged at high speed(10000-12000 rpm) for 10 hours, resulting in separation of DNA from remaining substances based on its density.
- EtBr is incorporated more into nonsupercoiled DNA than supercoiled DNA molecules resulting in accumulation of supercoiled DNA at lower density, and location of DNA is visualized under ultraviolet (UV) light.

Advantage: This method is used to extract DNA from bacteria.
Limitations:

- Greater amount of material source is needed.
- Time-consuming.
- Costly procedure due to long duration of high-speed ultracentrifugation.
- Complicated method.

Alkaline Extraction Method:
First introduced by Birnboim and Doly in 1979, this method is used to extract plasmid DNA from cells.

Sample s suspended in NaOH solution and SDS detergent for lysis of cell membrane and protein denaturation. Potassium acetate is then added to neutralize the alkaline solution, which results in formation of precipitate. Plasmid DNA in the supernatant is recovered after centrifugation.

Limitation:
Contaminations of plasmid DNA with fragmented chromosomal DNA.

Phenol-chloroform Method:
This method was introduced by Barker in 1998.

Procedures:
Lysis containing SDS is added to cells to dissolve cell membrane and nuclear envelope phenol-chloroform-isoamyl alcohol reagent is added in the ratio 25:24:1. Both SDS and phenol cause protein denaturation. While isoamyl alcohol prevents emulsification and hence facilitates DNA precipitation. The contents are then mixed to form biphasic emulsion that is later subjected to movement. This emulsion separates into two phases upon centrifugation, upper aqueous phase, composed of DNA and the lower organic phase, composed of proteins. Upper aqueous phase is transferred to fresh tube and the lower organic phase is discarded. These steps are further repeated until the interface between the organic and aqueous phase is free from protein. Later, sodium acetate solution and ethanol are added 2:1 or 1:1 ratio, followed by centrifugation for separation of DNA from the solution. The pellet is washed with 70% ethanol to remove excess salt from the DNA and the subjected to centrifugation for removal of ethanol. The pellet is dried and suspended in an aqueous buffer or sterile distilled water.

Advantages:
- Used to extract DNA from blood, tissue homogenate and suspension culture.
- Inexpensive and Gold standard method.

Limitations:
- Toxic nature of phenol and chloroform

DNA Quantification:
After DNA extraction, an accurate measurement of the amount and quality of DNA extract is desirable. When the correct amount of DNA is added to PCR, it results in the best quality within short duration of time. Adding less or more amount of DNA will results in a profile that is difficult or impossible to interpret. Quantity of DNA that can be extracted from a sample depends on the type ofmodel.

Classification of Quantification:
DNA quantification can be classified as follows:
Nonnucleic acid-based quantification methods.
- Microscopic and macroscopic examination.
- Chemical and immunological methods.
- DNA-based total genomic methods.
- Intact and degraded DNA-UV spectrophotometry.
- Intact vs degraded DNA-agarose gelelectrophoresis.

Real-time PCR, DNA-based target specific method
- Y chromosome DNA, mitochondrial DNA (mt-DNA), Alu repeat real-time PCR.
- Human autosomal DNA
- Multiplex real-time PCR.
- End-point PCR DNA quantification and alternative DNA detection methods.
- RNA-based quantification.

Visualization on agarose gel:
Advantages:
- It is relatively easy and quick method for assessing both quality and quantity of extracted DNA.
- Gives indication of size of extracted DNA molecules.

Disadvantages:
- Quantification is subjective.
- Total DNA obtained can be mixture of human DNA and microbial DNA and this can lead to overestimation of DNA concentration.

Ultraviolet Spectrometry:
Spectrometry is commonly used for quantification of DNA in molecular biology but has not been widely adopted by the forensic community.

Usually, DNA absorbs light maximally at 260nm;

this feature is used to estimate the amount of DNA extraction by measuring wavelengths ranging from 220nm to 300nm. With this method, it is possible to assess the amount of protein (maximum absorbance is 280nm) and carbohydrate (maximum absorbance is 230nm). If the DNA extract is clean, the ratio of absorbance should be between 1.8 and 2.

Disadvantages:

- Difficult to quantify small amounts of DNA.

Fluorescence Spectrometry:

Ethidium bromide or 4',6 diamidino-2-phenylindole can be used to visualize DNA in agarose gels. In addition of staining agarose gels, fluorescent dyes can be used as an alternative to UV spectrometry for DNA quantification. PicoGreen dye is commonly used because it is specific for double-stranded DNA as it has the ability to detect little amount of DNA as 25 pg/mL. **Disadvantage:**

- Nonhuman specific.

DNA Amplification:

There are eight DNA- and RNA- based techniques, but PCR and reverse transcription -PCR have been the predominant technique. PCR is the commonly used method of amplification of DNA. PCR amplifies specific regions of DNA template; even a single molecule can be amplified to 1 billion-fold by 30 cycles of amplification. DNA amplification occurs in cycling phase, which consists of three stages:

- Denaturation.
- Annealing.
- Extraction. Other methods are as follows:
- Nucleic acid sequence -based amplification method.
- Strand displacement amplification.
- Recombinase polymerase amplification
- Strand invasion-based amplification.
- Multiple displacement amplification.
- Hybridization chain reaction.
- Detection of the DNA-Amplified Products:

The following methods are used in forensic human identification:

- Autosomal short-tandem repeat (STR) profiling
- Analysis of the Y chromosomes
- Analysis of mt-DNA
- Autosomal single-nucleotide polymorphism (SNP) typing.

Impact of Genetic Identification in Justice:

Genetic testing using DNA has been widely applicable to the field of justice. This method is being used for the following:

- Identification of victims in disaster.
- Identification of accused and confirmation of guilt.
- Identification of persons who commit crimes or serial killers.
- Exculpation of innocent ones.
- Establishing consanguinity in complex cases

CONCLUSION:

- Forensic Analytical Chemistry has wide scope in the field of criminal investigation where it has helped in solving many cases.
- Forensic science has a rich but undervalued history and a richer potential ahead.
- Advancement in forensic science is ongoing process and each day introduced a new technique into the forensic world.
- Forensic Analytical chemistry are the most promising and rewarding career today.

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