FORENSIC LAB PROCEDURES

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There is always a need for laboratory services in the investigation of criminal deaths,cases of physical and sexual assaults,identification problems including cases of disputed paternity and detection of controlled drugs for example alcohol or heroin in the blood of a driver.

A medical practitioner needs to know the nature of poison ingested by a victim or other toxins such as pesticide,insecticide or heavy metal entered in the body of a worker in an industry or other residents of the vicinity.It necessitates analysis of blood and other body fluids or tissues to detect such substances.

FORENSIC LAB SYSTEMS

The forensic laboratory services in Lahore exists as a partial laboratory system,which is neither functionally independent nor fully developed.

Some of the teaching departments of medical colleges have been assigned additional medicolegal duties pertaining to their speciality.

PARTIAL LABORATORY SYSTEM

Forensic Science Examinations:

Designation	Nature of Work
Director Forensic Science Lab,Lahore	Ballistics
Director Bureau of Finger Prints,Lahore	Dactylography
Director Bureau of Handwriting,Lahore	Handwriting Analysis
Chemical Examiner Punjab,Lahore	Forensic Toxicology.

Forensic Medical Examination:

Designation	Nature of Work
Professor of Anatomy,KEMC,Lahore	Forensic Anatomy
Professor of Bacteriology,CCM,Lahore	Forensic Bacteriology
Professor of Pathology,KEMC,Lahore	Forensic Pathology
Professor of Entomology,CCM,Lahore	Forensic Entomology
Director,Blood Transfusion Services,Lahore	Forensic Serology.

FORENSIC LAB PROCEDURES

- For the identification of biological trace evidence,the primary tools have been the use of five senses and intelligent interpretation of the observed findings
- The examiner should adopt standardized procedures in their proper order
- The recommended strategy is that the evidence whether bones or stain should be followed by application of other special techniques

- The results of examinations should be tabulated and presented on a form that may be understood easily by both police and law courts
- Examinations should be treated as serious problems
- The findings should not be disposed off without instructions from the area Magistrate
- The evidence collected from the scene of crime is usually in the form of blood spilt on floors or other items such as furniture,weapon of offence such as knife or clothes

- Other important items could be semen,hair and fibers of non-biological origin on clothes or the bodies of the victims or assailant
- The purpose of such examination is to know the source from where evidence has come.

FORENSIC HISTOPATHOLOGY

- Subspeciality of forensic medicine
- Deals with macroscopic as well as microscopic examination of a part ,an organ or a portion of an organ having a defect,which is removed during medicolegal autopsy
- The examination provides information regarding evidence of disease which may have precipitated sudden death or may help in assessing the effects of drug,physical exertion and trauma including psychological stress on fatal outcomes

- The objective is generally achieved by noting morbid anatomical changes in the body organs supplemented with information derived from histology and other techniques adopted in the laboratory
- Specimens are preserved in 10% normal saline and sent under sealed cover along with autopsy report to the laboratory for further examination
- The specimen is subjected to both naked eye examination and other examinations such as histology and histochemistry.

NAKED EYE EXAMINATION

- Includes noting of changes in respect of size,color,consistency and condition of covering
- These findings are entered in the standard Performa of the report register
- In case only a portion of an organ bearing pathology is received and detailed gross examination is unrewarding,the tissue is processed straight away for histology,etc.

HISTOLOGICAL EXAMINATION

- Histological examination of the suspected area requires preparation of slides
- Tissue bearing pathology is located and cut out along with some healthy tissue for comparison
- Cutting should be done from the margin of the lesion to include some healthy area
- Selected tissue is fixed,section prepared and placed on slides and stained before examination

- Fixing of the specimen can be done manually or by using automatic tissue processor
- The choice of fixing agent depends upon the purpose for which the tissue is to be stained or preserved
- Buffered formal saline is a good fixative to suppress autolysis and to mummify the tissue
- Other fixatives are alcohol and acetone but they are expensive

- For fixation,the specimen is placed in a container having 10 % fixative solution and is kept dipped overnight
- Soft tissues like liver,spleen and brain are cut in 2cm thick slices and placed in a more concentrated fixative for better results.
- Use of automatic tissue processor has improved working.

Automatic Tissue Processing Unit;

It is a machine having a basket for holding tissue sections and several beakers containing different specific fluids.The basket carrying the tissue sections dips automatically in beakers for preset timings.The advantages of use of automatic tissue processors are efficiency and accurate fixation timing.

PREPARATION OF SECTIONS

There are two methods;

1) Formalin-fixed-paraffin-embedded sections;

These are prepared after embedding the formaline fixed tissue in paraffin.Since paraffin does not mix with water,the tissue must be dehydrated first and then cleared in a solution,which is miscible with paraffin such as alcohol.Paraffin blocks are placed at an appropriate place in the microtome for cutting into sections.The sections should not be more than 4 micrometer thick.Mounting of the section onto glass slide is done by smearing the slide with an adhesive solution and taking the section on it in the center by lifting the section floating in the microtome dish and leaving it to dry.

2) Frozen sections;

These are generally prepared when histological reporting is demanded within few hours.Formaline fixed block is washed with water,processed in degrading concentrations of alcohol and stored in liquid nitrogen.It is adequately frozen for 5-15 minutes using cryostat(coldtome)machine where temperature control is between -40 to -70 degrees Centigrade.Cryostat has built-in microtome to prepare 5 micron thick sections.These sections get fixed to the glass slides without adhesive solution by leaving them on the slide at room temperature.

STAINING

The slides bearing sections are stained either manually using hematoxylin and eosin stains or with the help of automatic tissue stainer(timer monitoring).All slides are scanned for relative comparison among the histological evidence of different sections from the organs of the same cadaver and gross and autopsy findings.

HISTOCHEMISTRY

- It provides the most sensitive and earliest demonstration of tissue changes and in most cases within 2-4 hours after onset of disease
- The procedure is extremely useful in the diagnosis of myocardial infarction.
- The rationale of the technique is to allow the surface of the tissue to be incubated in contact with a substrate containing a fluid (indicator).

- The dead or ischemic cells have a reduced or absent dehydrogenase activity and therefore these areas of section remain unstained.
- Findings observed on gross and microscopic examinations of all viscera should be entered on the prescribed Performa of the register by the forensic histopathologist in his own handwriting
- The conclusion should be based upon history,gross and microscopic findings

- Two copies,an original and a carbon copy should be prepared vide the number allotted to the case
- Original of the report is retained in the register and the carbon copy is dispatched to the medical practitioner who has requested the examination
- In case of a tumor,an additional copy of the report is sent to the Provincial Director,Tumor Registry.

STEAM DISTILLATION

It is a method for seperating volatile substances,which are insoluble in water from non-volatile impurities.It is applied to those compounds which have an appreciable vapour tension at the boiling point of water.

Steps involved;

i. Weigh the material
ii. Mince it finely
iii. Mix with water 3-5 times its volume
iv. Acidify with non-volatile acid (tartaric acid).The alkaloids are soluble in water only in the presence of acid
v. Subject to steam-distillation ,till volatile substances are carried over.

Technique;

The specimen is placed in a flask and the steam current from a small boiler is passed through it.The flask is set in sloping position and heated over a waterbath.The substance present in the specimen evaporates and goes along with the steam in a water condenser where it gets collected in the receiver.The distillate is transferred to a seperating funneling which the liquid,which is not miscible with water,gradually forms a separate layer.It can now be removed easily and subjected to specific tests for identification of various volatile substances such as alcohol,cyanide,phenol,etc.

MICRO-DIFFUSION ANALYSIS

- It is a rapid method of isolation and detection of volatile poisons and gases including acetaldehyde,carbon monoxide,cyanide,ethanol,methanol,fluoride and halogenated hydrocarbons
- A simple micro-diffusion apparatus consists of a small porcelain dish having two compartments,a central well surrounded by a peripheral wall and a ground glass cover-plate . The outer wall of the peripheral well is slightly higher than the partition wall

- The sample such as blood,urine or tissue homogenate is placed in the outer well and the color reagent,which is either an absorbent or solvent,is placed in the inner well
- The dish is covered with cover-plate and sealed with an inert viscous material
- The volatile poison on gently heating the dish will diffuse from the sample in the outer well into the atmosphere of the dish and finally will be entrapped by the reagent in the inner well.
- It produces specific color change in the inner well indicating the type of substance.

STAS-OTTO METHOD

Principle;

Glucosides and alkaloids are soluble in alcohol and thus can be seperated from proteins and fats,by alcohol.The glucosides being soluble in solvents like chloroform and ether and alkaloids being readily soluble in water in presence of small amounts of acid can be extracted from them.Alkalis make the alkaloid insoluble and then with organic solvents one can easily extract them.

Disadvantages;

Though the test seems simple yet in actual practice it is rather tedious,lengthy and has a definite risk of loss of poison due to so many manipulations.

Procedure;

- The test involves a series of filtration and extraction
- The material is weighed,minced finely,mixed with two times volume of alcohol and acidified with tartaric acid
- This mixture is shaken and passed through many series of filtration and extraction over a long period to get rid of fats and proteins
- The residue contains organic glucosides and alkaloids

- After filtration,it is mixed with an organic solvent like chloroform or ether or their mixture(3:1).The aqueous solution is shaken with organic solvent gently to avoid formation of an emulsion.Shaking is done in a seperating funnel.
- Aqueous solution and chloroform gets seperated as chloroform settles down in the lower layer
- The chloroform is allowed to evaporate and remaining aqueous solution is made alkaline to liberate the free alkaloids

- Identification of glucosides and alkaloids can now be done with specific tests important being color reactions,chromatography,ultra-violet spectrometry and Neutron-activation analysis.

COLOR REACTION METHOD

- It is a technique in which the substance tested for is acted upon by a reagent producing an observable color or changing the color of the reagent in the chemical procedure
- The reaction indicates the group to which the poison belongs
- The procedure is both easy and rapid
- Technique involves mixing of the extracted poison with specific reagents.Further,use of reagents of increasing specificity narrows the identity of the poisons

- Confirmation of the identity of the drug can be done by color test known to be specific along with control test for comparison.

CHROMATOGRAPHY

- it is a diverse group of techniques used to separate mixtures of substances
- It is based on the principle of differences in the relative affinities of substances for two different media.Of the two,one is a moving fluid called the mobile phase and the other,a porous solid or gel or a liquid coated on a solid support,called a stationary phase.
- Procedure; A variety of attractive forces between stationary phase and substances to be seperated leads to the selective retardation and redistribution of substances relative to the mobile phase

- The attractive forces will in turn depend on the solubility and size of the particles
- The speed at which each substance is carried along by the mobile phase depends on its solubility in a liquid mobile phase or vapour pressure in a gas mobile phase and on its affinity for the sorbent

- Sensitivity;

It is a highly sensitive procedure widely used for both qualitative and quantitative detection of drugs such as alkaloids and their metabolites.

- Types;

a) Absorption chromatography
b) Affinity chromatography
c) Column chromatography
d) Gas chromatography(Gas-gas/Gas-liquid/Gas-solid)
e) Gel filtration chromatography
f) Gel permeation chromatography
g) High pressure liquid chromatography
h) Ion exchange chromatography
i) Liquid chromatography
j) Paper chromatography
k) Thin layer chromatography

THIN LAYER CHROMATOGRAPHY

- It is the frequently used procedure
- Procedure; A suitable material is required for the preparation of a thin layer.It may be silica gel,kieselguhr gel or cellulose powder.A slurry is produced by thoroughly mixing 30 grams of selected material with 60 ml of water.The chromoplate is then prepared by spreading the thick aqueous slurry rapidly and uniformly by a special applicator.Machines are also available for spreading the thin layer.The coated plate is air dried at room temperature for 15 minutes and then in oven at 100-110 degrees Centigrade for 30 minutes,to remove even the last traces of moisture.

- The optimum thickness is 0.25 mm for routine analytical work
- Advantages;
  - Economical
  - Less time consuming
  - Does not involve sophisticated and complicated apparatus
  - Highly sensitive(can detect concentrations of 10-20 microgram)
  - Can withstand the action of most chemicals.

SPECTROSCOPY

- Principle;

Absorption of radiation is characteristic of all molecules.Wavelength of radiation may vary from infrared to ultraviolet rays.The most useful wavelength for toxicological analysis at present is ultraviolet.The interactions between the radiation and a chemical substance depends upon the energy of radiation,which is indicated by wavelength,and structure of the molecule of a substance.The interaction is absorption of light from a source of radiation and then emission of light.

ELECTROPHORESIS

- It separates mixtures of proteins by forcing them across a gel-coated plate under the influence of an electric potential.
- Due to variations in charge and size, proteins will move across the plate at different speeds
- After completion of the electrophoresis run, the separated proteins are stained with suitable developing agent for visual observation
- Thus, characteristic band patterns are obtained that are related to enzyme type present in the blood.

- Likewise,a mixture of DNA fragments can be seperated by gel electrophoresis by taking advantage of the fact that the rate of movement of DNA across a gel-coated plate will depend on the molecule’s size.Smaller DNA fragments will move at a faster rate along the plate than will the larger DNA fragments.

RADIOACTIVATION TECHNIQUE

It relies on the study of the behaviour of the electron around the nucleus.The properties of any nucleus can be studied by making the nucleus unstable.Highly specialized instruments make the nucleus unstable which make it possible to study its properties.

DETECTION OF INSECTICIDE COMPOUNDS

- This has not been fully achieved yet
- Many drugs have anti-cholinesterase activity and in the absence of any identifiable drug,an intense anti-cholinesterase activity is indicative of an organophosphorus poison
- There are other tests technique to test for chloride and phosphorus

- The procedure consists of taking 50 grams of minced viscera preferably liver and fat because insecticides are deposited in fat.The whole contents are covered by hexane.The flask is fitted to reverse condenser and contents are heated on a very low flame.This continuous extraction is carried out for 24-48 hours.The contents are then cooled and subjected to frequent washings with distilled water to obtain salt-free hexane layer.The hexane is evaporated and contents are then read either in spectrophotometer in appropriate wavelengths along with standard samples of insecticides or the residue is transferred in a platinum cup in which sodium carbonate has previously been added.Contents of platinum cup are reduced to ashes at 65-75 degrees Centigrade in a furnace.The contents of the cup are allowed to cool and then treated with strong nitric acid and filtered. It is then tested for chloride and phosphorus.

- Paper chromatography, high-performance liquid chromatography or gas-liquid chromatography can also determine the insecticides.

COLLECTION & PRESERVATION OF SPECIMENS

COLLECTION OF SPECIMENS

- It is essential in the cases of intoxication,sexual assaults and any other case requiring collection of blood and other samples for investigation purposes
- The success of the investigation depends upon the proper collection and preservation of the right material from the body of the victim or assailant
- The procedure of collection and custody of medicolegal specimens differs from that of collection of samples in clinical practice.

- In clinical practice;both the patient and the laboratory are placed in one building.Either the patient is referred to the laboratory or the technician comes to the ward to collect the samples from the patient.There is no chance of substitution,except an error of labeling,as interest of both is the same,that is,to achieve correct results quickly.

- In medicolegal practice;the place of collection of the samples and the place of examination are miles apart and the interests of both parties to interfere with them in form of addition or substitution are immense.Such an interference may take place at the place of collection,during transit of the sample to the laboratory and finally in the laboratory,where samples are processed and examined.The persons possessing the evidence form the chain of custody and a short chain is preferable.All are required to certify the extent of handling.The evidential material should be handed over only after obtaining a receipt.

INSTRUCTIONS FOR COLLECTION OF SPECIMENS

- General instructions
- Special instructions

GENERAL INSTRUCTIONS

1) The source of the specimens should be beyond doubt
2) The authorized staff should only collect the specimens
3) After collection,the specimens should be stored in a freezer temperature till processing
4) The container of the specimen should be impervious,clean and free from contamination
5) The size of the container should be adequate in relation to the specimen
6) The mouth of the container should be appropriately wide to admit the specimen
7) The stopper of the container should be screw-type to avoid leakage

8) Every container should bear a label with the following enteries;

- Name of specimen
- From whom and from where collected
- Type of examination required
- Name and designation of the person who collected specimen
- Date and place of collection

9) The specimen should be sealed properly before dispatch

10) A forwarding letter containing the request of examination required in the laboratory should be sent along with these specimens

11) If the samples are more than one,then each specimen should be identified with an identity number and the schedule showing identity numbers allotted to each specimen should be enclosed with the forwarding letter
12) The transportation of specimens to the laboratory should be done through the police official responsible for the investigation of the case
13) If the specimens are to be sent by post or rail,they should be either registered or sent through prepaid railway parcel
14) At the time of receipt of samples in the laboratory.it should be ensured that the seals are intact and the related papers are complete in all respects
15) The analyst should sign the register to maintain the chain of custody

16) The registration of the container or sample should be done with the following enteries;

- Serial number of the year
- Date of receipt of the sample
- Bio data of the victim
- Date of medicolegal/autopsy examination
- Number and the name of the sample
- Place from where dispatched

SPECIAL INSTRUCTIONS

These are applicable to the individual specimens and they vary with type of specimen and the objective of collection, whether chemical analysis,histopathology,serology or as a museum specimen.The instructions for various types of specimens are as follows;

BLOOD

- Blood is either collected for determination of its source or for estimation of a drug or poison
- When it is collected from the scene of crime,the quantity should be sufficient(it should be about 10 cc.)
- It is collected from a peripheral vein of the subject subject
- Blood for grouping in cases of paternity disputes should be collected simultaneously from the child and the parents who should verify their identity
- Skin should not be cleaned with spirit while taking a sample for alcohol estimation

- In dead bodies,1oo cc. of blood is collected from the right heart.It is the specimen of choice in deaths due to carbon monoxide poisoning
- The container should be special having a stopper to avoid loss of poison by evaporation.It should be chemically clean.No preservative is used
- Delay in dispatch should be avoided in the cases of poisoning with alcohol and carbon monoxide and bacteriological infection.The samples must reach the laboratory within few hours and must be examined immediately,before they start decomposing.

SALIVA

- It is required for grouping purposes
- After thorough mouthwash,about5 cc. of saliva is collected by instructing the subject to loosen his mouth and let saliva dribble in a test tube.If necessary,the mucous membrane may be tickled with a glass rod
- The food particles that are present in the saliva should be separated and the enzyme amylase inactivated before dispatch to the laboratory.The enzyme is inactivated by indirectly heating the test tube containing the sample after placing it in the water bath for about10-15 minutes

- After cooling the specimen is centrifuged at 2000-3000 revolutions per minute for 10 minutes.The food particles get settled at the bottom of the tube and the clear supernatant fluid is collected in a clean test tube,which is then sent.

SEMEN

- It is generally collected on a slide or on a swab to detect spermatozoa in cases of sexual assaults
- When present on clothes,the body of the victim or assailant or at places other than the vagina and rectal canal.it is usually in dried state.It should either be scraped with a dry scalpel or collected first on a glazed paper and then passed into a special container avoiding loss of the sample,or the area may be moistened with saline and the material taken on a guaze/swab attached on the end of an applicator

- From the vaginal or anal canal, it is taken before taking the urine specimen and before digital examination
- The material is received on a cotton swab attached to an applicator with the help of a suitable sized,sterile,non-contaminated speculum. Careful withdrawal is necessary to avoid loss of specimen due to rubbing on the inner side of speculum

- Three swabs are generally prepared of which two swabs are air dried and preserved in separate test tubes for dispatch to the laboratory for further examination. While it is still wet,the third swab is used to prepare three slides,which should be both clean as well as clear.
- Immediately after withdrawal from the vagina or anal canal,it is gently rubbed only once over the central portion of the slides.The film should be thin.Slides are air dried.The best two are packed having smeared surfaces facing each other with two matchsticks placed between them near the free margin of the slides.the slides should be wrapped with adhesive tape to avoid displacement and loss of specimen.

- Both swabs and the slides, duly labelled, are placed in a container, which is duly sealed.

THE END.