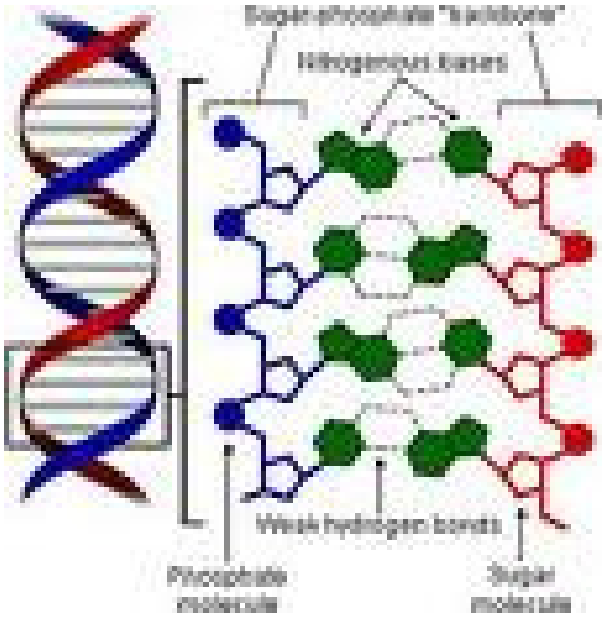
WORKSHOP FOR MEDICAL OFFICERS

Reading Material



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Collection of Evidence for DNA Fingerprinting

Crime is as old as human civilization. The primary aim of forensic examination is to collect the evidence that may help to prove or disprove a link between individuals and / or between individuals and objects or places. DNA ie. Deoxyribonucleic acid is the vehicle of generational transference of heritable unit. Forensic DNA analysis or DNA Fingerprinting helps in establishing an association between biological evidence and its source ie a suspect, victim, crime scene or weapon . In analysis, specific genes in the questioned item are compared with those in the known specimen.

Collection, preservation and handling are the integral part of DNA fingerprinting."Medico legal" term incorporates the basis of two sister professions ie medicine and law. The medicolegal experts can provide a link between these two professions for smooth effective functioning in a scientific manner. Area where medicolegal experts may have to collect specimens for DNA fingerprinting are-

- Paternity disputes
- Sexual offences
- Heinous crime
- Mass Disaster

Earlier forensic scientists have been employing gene coded polymorphic products to link a suspect to the crime . They have been using different techniques like blood grouping, HLA typing, isozyme grouping etc. in the biological material recovered at the scene of crime

for investigation. These tests are based on proteins, which get highly degraded with time. Thus there was necessity for identification of biological material, which on the one hand is very stable and on the other hand is so variable that it is individual specific. The discovery of DNA fingerprinting caught the imagination of forensic scientists to link with certainty the origin of biological material.

DNA fingerprinting has had a major impact on the criminal justice system and law during the last decade of the 20 th century. It has been employed in criminal law to help prove guilt or innocence , in family law to prove paternity , and in immigration law to prove blood relationships or to establish citizenship. Its usefulness as a human identification tool is evident. Accordingly in recent years our legal system has given DNA fingerprinting the credibility that nature has given it as the blue print of life.

WHAT IS DNA?

DNA or deoxyribonucleic acid is the genetic material and the carrier of genetic traits. A part of DNA of various individuals is the same, but another part differs from individual to individual (except in the case of identical twins). Portions of DNA structure of certain genes are as unique to each individual as fingerprints. Alec Jeffreys and his colleagues who were responsible for these revelations named the process for isolating and reading these DNA markers as "DNA Fingerprinting". In forensic examination narrowing the source of biological material i.e.

individualization remains an elusive goal. DNA fingerprinting has brought forensic scientists to the brink of this goal. It provides a virtually foolproof method of establishing identity including parentage of an individual.

The human body is made up of 60 trillion cells. In cell are strands of genetic material called chromosomes. Each human cell (except red. blood cell) contains 23 pairs of chromosomes-23 from the father and 23 from the mother. Each chromosome consists of a long chemical molecule of DNA complexed with proteins. DNA is a polymer ie, a chain of small repeated sub units found in the nuclei of all human cells (except red blood cells) and sub cellular structure mitochondria. The structural unit of DNA is deoxyribonucleotide. A segment of DNA , which instructs the body cells to make proteins that determine everything from eye colour to our susceptibility to resist diseases is the fundamental unit of heredity called **gene.** However function of 95 percent of DNA is not yet understood and is known as junk DNA. In which lies the mystery of individualization, ie, why one person is so different from another. DNA fingerprints of different unrelated individuals are different while related individuals show a high coefficient of similarity.

DNA is made up of three components- (i) nitrogen bases (ii) carbohydrates (deoxyribose sugar) and (iii) phosphate. There are four types of nitrogen containing bases in DNA—Adenine(A), Guanine(G),

Thymine(T) & Cytosine(C). Adenine and Guanine are called purine and Thymine and Cytosine are pyrimidine. Sugar along with phosphate groups form the backbone to which these bases are linked.

The double helix model of DNA was proposed by Watson and Crick (1953). The two strands are linked by hydrogen bonds between pair of base pairs. Adenine on one strand always pairs with thymine via two hydrogen bonds (A=T) and guanine with cytosine via three hydrogen bonds (G≡C). Out of 3.3 billion base pairs that make a human being, approximately 3 million differ between any two individuals ie only one – tenth of a single percent of DNA differs from one person to the other.

DNA FINGERPRINTING

DNA fingerprinting or DNA typing or DNA profiling is a technique that detects DNA pattern unique for every individual. It is a complex process of analysis of some highly variable regions of DNA. It involves the intersection of several scientific disciplines, including molecular biology, genetics and statistical analysis. It is a reliable molecular tool for the law agencies for resolving certain critical issues in crime investigation.

Portions of the DNA molecule of the non coding region contain sequences of letters that are repeated numerous times (tandem repeats) and offer a means of distinguishing one individual from another through

DNA fingerprinting. Within the world's population there are numerous possibilities for the number of times a particular sequence of base letters can repeat themselves on a DNA strand. The stuttered region of DNA where a short sequence of bases, typically 20 base pair long, is repeated over and over again and is called "**minisatellite**". In case of **"microsatellites"** a short sequence of DNA, about 3 to 7 base pair long, is repeated. Forensic scientists scan 15 different regions that vary from person to person and used the data to create a DNA profile / fingerprint of that individual. The probability that another person has the same DNA profile for a particular set of regions is extremely low. In criminal cases the scientists extract the DNA from different samples and analyze it for the presence of a set of specific DNA regions (markers). The following DNA technologies are used in forensic investigations –

(i)Restriction Fragment Length Polymorphism (RFLP)

The methods used in DNA fingerprinting are conventional techniques of molecular biology. The first technique that was adopted for forensic DNA analysis was RFLP.

Unfortunately, the RFLP technique requires a greater amount of better quality DNA than the newer PCR based techniques. In addition forensic evidence is often old, degraded and of limited quantity where RFLP is sometimes not possible.

(ii)PCR Analysis

Polymerase Chain Reaction (PCR) is used to make millions of exact copies of DNA from biological samples. DNA amplification with PCR makes possible DNA analysis on biological samples as small as few skin cells.

(iii) STR Analysis

The latest method of DNA fingerprinting, short tandem repeat (STR) or microsatellite analysis has the potential for a higher discrimination and also reduces the amount of time to obtain results. It also requires a sample size smaller than that needed for RFLP methods. STR technology is used to evaluate specific regions (loci) within nuclear DNA. STRs are locations (loci) on chromosome that contain short sequence elements that repeat themselves within the DNA molecule. The repeat sequence as mentioned earlier is 3-7 bases and the entire strand of STR less than 400 bases in length. Hence, STRs are less susceptible to degradation and may often be recovered from bodies or stains that have been subjected to extreme decomposition. The Federal Bureau of Investigation (FBI) uses a standard set of thirteen specific STR regions. The odds that the two individuals will have the same 13 loci DNA profile is about one in one billion. The following steps are involved in STR analysis -

- Extracting and purifying DNA from biological evidence.
- Amplification of selected genetic markers through PCR ie polymerase chain reaction.
- Visualizing the fragments and genotyping.
- Statistical analysis and Interpretation.

(IV) Mitochondrial DNA analysis (mt DNA)

In the investigation of cases that have gone unsolved for many years mt DNA is extremely valuable . Nuclear DNA must be extracted from samples for RFLP, PCR and STR, however mt DNA analysis uses DNA extracted from mitochondria (another cellular organelle). Older biological samples that lack nucleated cellular material for example hair, bones and teeth can be analysed with mt DNA. All daughters have the same mt DNA as their mothers because mitochondria of each embryo comes from mother's egg cell- father's sperm contributes only nuclear DNA. Comparison of mt DNA profile with profile of a potential maternal relative can be an important technique in solving missing persons identity and maternity disputes.

(V) Y-chromosome Analysis

The Y-chromosome is passed directly from father to son and hence the analysis of genetic markers of Y-chromosome is especially useful for analyzing biological evidence involving multiple male contributors.

The advantages of DNA Fingerprinting can be summarized as follows -

- Discrimination potential is very high hence individuals as close as brothers or sisters (except monozygotic twins) can be identified.
- DNA profiling is feasible even from degraded or very minute amount of biological material (at times invisible to the naked eye) because of high sensitivity.
- DNA molecule is very stable.
- DNA profiling can be done from any biological material and is not restricted to any specific organ/area of the body, unlike dermal fingerprinting.
- Determination of species of origin and gender is feasible.
- DNA fingerprinting leads to better administration of justice and increased public confidence in the Criminal Justice System.

APPLICATIONS

(A) Civil Cases

1. In proving paternity/maternity
2. Solving cases of switched babies
3. Determining immigration status
4. Identification of victims of accident, fire, natural disasters etc.
5. Delineating family lineage

(B) Criminal Cases

1. Solving murder cases
2. Linking victim and culprit in sexual offences
3. Identification of mutilated bodies/ Skeletons/ source of tooth pulp
4. Sexing biological material
5. Solving crimes related to animals
6. Solving crimes related to plants

LEGISLATIONS

Cr.P.C and DNA Testing

(i) Section 53, Cr.P.C.-

The title or marginal notes of section 53 is "Examination of accused by medical practitioner at the request of police officer".

No doubt the provisions of section 53 are applicable during investigation and when a person is in custody after his arrest on a charge. As per the section -

1. When a person is arrested on a charge of committing an offence of such a nature and alleged to have been committed under such circumstances that there are reasonable grounds for belonging that an examination of his person will afford evidence as to the commission of an offence, it shall be lawful for registered medical

practitioner, acting at the request of a police officer not below the rank of sub inspector, and for any person acting in good faith in his aid and under his direction, to make such an examination of a person arrested as is reasonably, necessary in order to ascertain the facts which may afford the such evidence, and to use such force as is reasonably necessary for that purpose.

2. Whenever the person of a female is to be examined under this section, the examination shall be made only by, or under the supervision of, a female registered medical practitioner.

[Explanation- *In this section and in sections 53-A and 54,-*

(a) "examination" shall include the examination of blood, blood stains, semen, swabs in case of sexual offence, sputum and sweat, hair samples and fingernail clipping by the use of modern and scientific techniques including DNA profiling and such other tests which the registered medical practitioner thinks necessary in a particular case;

(b) "registered medical practitioner" means a medical practitioner who possess any medical qualification as defined in cause (h) of section 2 of the Indian Medical Council Act 1956 and whose name has been entered in a State Medical Register.]

(ii) Section 53A, Cr.P.C.-

Section 53A relates to specific accused who has been arrested for charges of rape or its attempt and there are reasonable grounds for believing that the examination of his person would afford evidence, the accused may be examined medically at his arrest and even a reasonable force may be used, which is necessary for the purpose for such examination. The examination of the accused has been subjected to the condition, that "there are reasonable grounds for believing" that examination would afford evidence.

(iii) Section 164A, Cr.P.C.-

The provision of section 164A relates to examination of a victim of rape, including taking a sample from her person, with her consent for "DNA profiling". For a very long time victims of any kind of assault and injury, including victims of rape had been medically and forensically examined and the examination report prepared during such examinations was produced in courts as evidence.

As per the section, the registered medical practitioner, to whom such woman is sent shall, without delay, examine her person and prepare a report of his examination giving the following particulars, namely-

(i) the name and the address of woman and of the person by whom she was brought;

(ii) the age of the woman;

(iii) the description of the material taken from the person of the woman for DNA profiling;

(iv) marks of injury, if any, on the person of the woman;

(v) general mental condition of the woman; and

(vi) other material particulars in reasonable details.

It seems that there is sufficient law ii existence to deal with DNA evidence, in the state it is at present. Where peculiar situations arise the courts have inherent powers to deal with such situations as they have been doing now.

MEDICOLEGAL ASPECTS

A. MEDICO LEGAL ASPECTS OF SEXUAL OFFENCE

Collection of Forensic DNA Evidence

The rationale for collecting forensic evidence is to link a suspect to the victim of the crime. In order to collect suitable forensic evidence, the health worker must understand the types of evidence that may be present. The selection of the sample taken should be directed, in part, by the hospital or clinic. Specimen collection should be performed as soon as possible in order to minimize loss and degradation of the sample (e.g. loss of semen from drainage, douching, etc.).

Control sample

A general principle when collecting evidence for forensic purposes is to collect a control or reference sample at the same time as any evidence or test sample, which may be connected to the crime. A control sample is a known sample, for example, blood, hair or tissue, which may be compared with a sample

obtained from the crime scene (e.g. semen from the vagina of a rape survivor or blood-stained clothing).

Storage of samples and preservation of chain of evidence

Steps to ensure that the evidence is reliable include: using appropriate containers or bags; proper labeling; proper sealing (e.g. use of tamper-proof seals); secure storage; and maintenance of the chain of custody. This will result in the forensic analyses being acceptable to the court. The samples should be initialed and dated by the collector and handed to the investigating officer who should sign for their receipt. If there is any detay in handing the samples to the investigating officer or laboratory, the samples should be securely stored, preferably in locked facility with restricted access until they are handed over.

Preservation of samples

Wet samples (e.g. clothing) should be air dried to prevent growth of bacteria and fungi which may degrade DNA making the sample unsuitable for analysis. Samples containing biological evidentiary material such as DNA should be stored in a cool dry environment or refrigerated to prevent putrefaction (decomposition) which may render the sample unsuitable for analysis. Care must be taken to avoid contamination of the sample by the health worker who may inadvertently add material such as hair, blood or bacteria during collection, preservation, or handling of the sample.

Taking of swabs

The purpose of taking swabs is to collect samples of any body fluids that may have been deposited on the outside of the rape survivor's body or on any interior orifice which may assist to connect the assailant with the alleged assault through DNA analysis of the samples. The areas of the body from which the samples are taken may help to corroborate the survivor's version of the events that are alleged to have occurred. These areas include the inside of the mouth (for

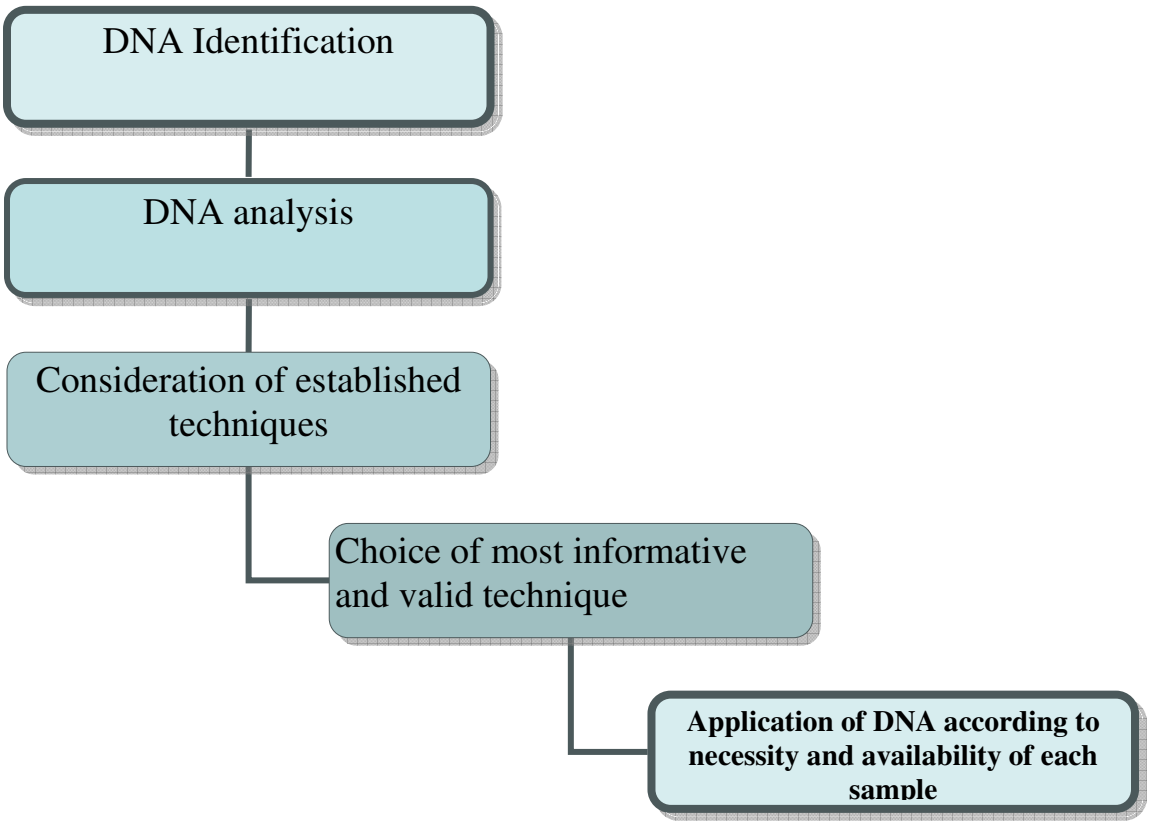
evidence of semen) or the exterior of the vagina (for evidence of saliva) if oral sex was performed; the areas around the aureole or breast if they were bitten, licked or sucked by the assailant (for evidence of saliva); the face, neck, cheeks or lips of the mouth if they were kissed or licked by the assailant (for evidence of saliva); any areas of the body, head or limbs on which the assailant any have ejaculated (for evidence of semen); and under the fingernails where the survivor may have scratched the assailant.

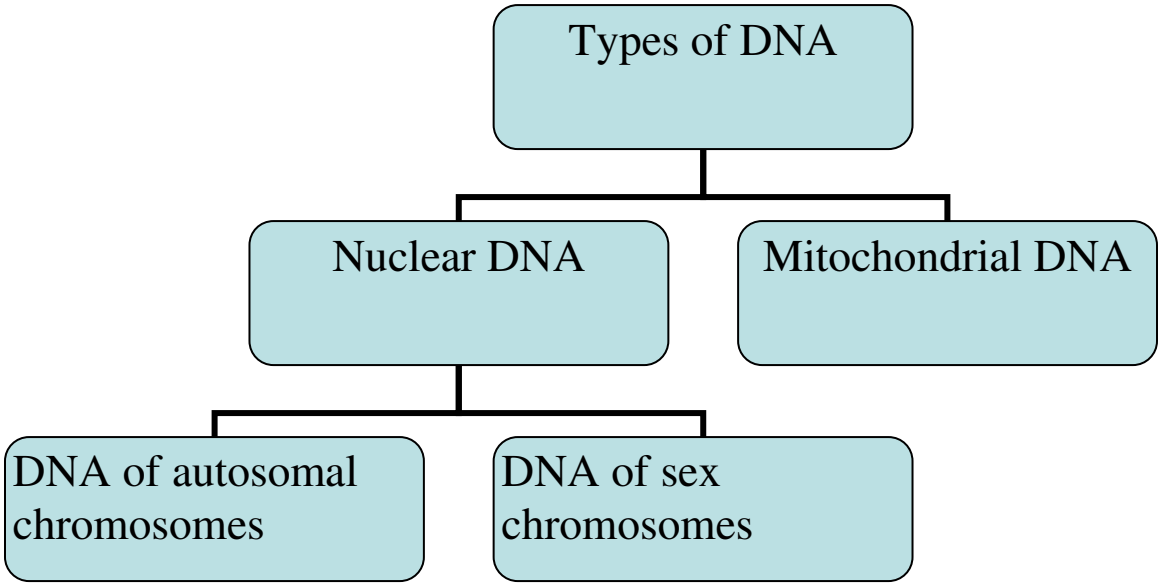
Body fluids

Body fluids such as blood or semen are likely to remain stuck on the rape survivor's skin if they become air-dried and are not washed off or absorbed by materials such as clothing or bedding. Saliva may evaporate but will leave cells from the oral mucosa behind. The length of time that spermatozoa will last in a survivor will depend upon where it is deposited on the body.

B. MEDICOLEGAL WORK IN MASS DISASTER

There is no justification from the medico-legal standpoint not to follow all scientific procedures for the recovery, transfer, identification, and final disposal of the remains of disaster fatalities. A select group of experts who are experienced in these procedures should oversee the process. However, in situations where experts are not available, the community physician should take leadership and make use of all available resources to carry out the job.


DNA Identification
DNA analysis
Consideration of established techniques
Choice of most informative and valid technique
Application of DNA according to necessity and availability of each sample


Types of DNA
Nuclear DNA
Mitochondrial DNA
DNA of autosomal chromosomes
DNA of sex chromosomes

C. MEDICOLEGAL WORK IN PARENTAGE DISPUTE

Presently blood is the classic, conclusive sample for determining DNA. Blood can be obtained by capillary or venous puncture.

The following table lists the range of forensic specimens that are typically of interest for a medicolegal expert along with appropriate collection techniques and comments on their relevance.

Table 1.Forensic Specimens

Site	Material	Sampling	Sampling Instruction
Anus (rectum)	Semen	Cotton swabs and microscope slide	Use swab and slides to collect and plate material; lubricate instruments with water, not lubricant.
	Lubricant	Cotton swab	Cotton swab, dry swab after collection.
Blood	DNA (Victim)	Appropriate tube	Collect 2 - 5 ml of venous blood in EDTA vial or prepare stain on FTA paper.
Clothing	Adherent foreign materials (e.g. semen, blood, hair, fibers) .	Paper bag(s)	Clothing should be placed in a paper bag(s). Collect paper sheet or drop cloth. After air dry wet items should be bagged separately.
Genitalia Male & female	Semen	Cotton swabs and microscope slide	Use separate swabs and slides to collect and plate material collected from the external genitalia, vaginal vault and cervix; lubricate speculum with water not lubricant or collect a blind vaginal swab.
Hair	Comparison of hair found at crime scene	Sterile container	Cut approximately 20 hairs and place hair in sterile container.
Mouth	Semen	Cotton swabs, sterile container	Swab multiple sites in mouth with one or more swabs. To obtain a sample of oral washing, rinse mouth with 10 ml water and collect in sterile container
	DNA (victim)	Cotton swabs	

Nails	Skin, fibers, blood etc. (from assailant)	Sterile toothpick Or similar or nail scissors/clippers	Use the toothpick to collect material from under the nails or the nail(s) can be cut and the clippings collected in a sterile container.
Sanitary pads/ tampons	Foreign material (e.g. semen, blood, hair)	Sterile container	Collect if used during or after vaginal or oral penetration.
Skin	Semen	Cotton swab	Swab sites where semen may be present.
	Saliva (e.g. at sites of kissing, biting or licking), blood	Cotton swab	Dry swab after collection.
	Foreign material (e.g. vegetation, matted hair or foreign hairs)	Swab or tweezers	Place material in sterile container (e.g. envelope, bottle)

Table 2. Collection of Forensic DNA Evidence

SPECIMENS / SAMPLES	COLLECTION	PURPOSE
Clothing	• Air dry wet or blood stained clothing (do not use extreme sources of heat e.g. blower, hairdryer etc.) • Once dried, place in paper bags (not plastic) • Do not store wet clothing. • Ensure packaging is properly labeled and delivered to laboratory as early as possible. • If delivery is to be delayed, store in a secure cool dry place.	• Identification of assailant using semen, blood or saliva stains or hair on clothing • To show corroborative evidence of force used eg. Torn clothing • To identify place where the crime was committed
Semen	• Semen on clothing should be treated as above. Collection of semen from body surface, mouth, anus, genitalia should follow the protocol given below- • A smear on glass slide by rolling a swab over a slide with out rubbing it as the latter may cause the spermatozoa to break and thereby give a false negative result. The slide is then air dried. • **Fixative** should not be used • All samples should be properly sealed, packaged and labeled.	• Identification of assailant • Confirmation of samples of semen

Blood	• Prepare stain on FTA paper; or • At least 2-5 ml blood should be collected as a control sample in EDTA vial. • Blood- stained clothing and objects should be treated as described above. • Separate items that are blood stained should be packaged individually and labeled and treated as above.	• To ascertain if the sample is from assailant or survivor, is with consent to intercourse/ defend her self.
Pubic or Head hair	• Hair sample should be collected using clean sterilized forceps • Individual hairs or clumps of hair should be separately packaged, • Hair with root tissues, or mixed with blood, body fluids or other tissue must be carefully collected to retain the integrity of the samples. • Hair mixed with wet fluids should be air dried as described above • Control sample of body, scalp, auxiliary and pubic hair should be taken, • Ideally by plucking and not by cutting the tips to obtain hair roots that contain adequate DNA analysis.	Identification of assailant vs. survivor.
Bones	• Wash with running water then air dry, place material in sterile cloth bound envelope/ wrap in paper or cloth.	Identification of body.

Table 3- DNA Content of Tissues

Source	DNA content (approximate)
Amniotic fluid	65 ng/ml ($1X10^4$ cells/ml at 16 weeks gestation)
Blood	40 µl (1 µl = 4 $X10^3$ to 11 $X10^3$ WBC, 1 WBC= 6.6 pg DNA
CVS	8 µg/mg
Hair roots	250 ng/plucked hair root
Liver	15 µg/mg
Muscle	3 µg/mg
Sperm	3.3 pg/cell

BASIC DO's & DONT's

Do's:

GENERAL (WITH SEXUAL ASSAULT VICTIM)

- Make sure you understand your own attitudes and feeling about sexual assault
- Let the victim know you believe her
- Let the victim know she survived, and that is not failure but success
- Encourage the victim cry, yell or talk
- Listen

TECHNICAL---

- To establish identity of deceased from skeletal remains, always collect intact long bones (femur, humerus)/ molar teeth in duplicate.
- Preserve tissue, foetus and other similar samples in 0.9% DNS and keep it in refrigerator for a short period if there is any delay in forwarding the sample to the laboratory.
- Always wrap stained clothes and fabrics in paper sheets and pack in cotton cloth or aerated container.
- If there are more than one sample, pack them separately.
- From dead body always take two or more types of samples in duplicate.
- Submit samples in laboratory without any delay.
- Always use disposable gloves, mask, syringes, dropper, blade, scissors for handling and collecting samples.

Don'ts:

GENERAL (WITH SEXUAL ASSAULT VICTIM)

- Do things for the victim without asking her first
- Get angry with victim
- Blame the victim
- Boss the victim around
- Rant and rave at the offender
- Try to make the victim believe the sexual assault was not serious

TECHNICAL-

- Never prefer to collect the clavicle bone.
- Never use formalin to preserve tissue and bones.
- Do not pack clothes/ garments, stain and swabs in wet condition.
- Never dry stains, swabs in direct sunlight by using heater, hot air blower etc.
- Never use cork/lid in vials while packing swab of semen, blood and other body fluids.
- Do not send completely burnt/ broken bones, burnt or singed hair.
- Never use polythene bag as packing material for biological evidence.

GUIDELINES

A. Evidence collection in sexual offences

Technical-

Step1: Oral specimen

Collect seminal fluid in the oral cavity for DNA analysis in cases where there is suspected oro-genital contact.

Step2: Collection of panties and sanitary pad

Collect the panties worn by the survivor during or after the incident. The sanitary pad must not be removed if it is attached to the panties. If the sanitary pad was detached at the time of medical examination, the sticky side of sanitary pad must be covered by waxed sheet to prevent the pad from sticking to the paper collection bag.

Step 3: Evidence of the patient's body

Collect and preserve any physical or biological evidence that may be present on patient's hair, skin or fingernails and to collect additional foreign debris. The site to be sampled is determined by asking the patient, by examination and by use of an ultraviolet light source, which will reveal stains that are invisible in normal light. The swab should be placed in dry rack of the swab guard box. If the bite marks are present they should be photographed after taking the necessary swab.

Step 4: Pubic hair

Comb to obtain any loose hair or debris that may help in identifying the assailant. Any matted hair may indicate the presence of blood or semen and should be cut over another sheet of catch paper so that it falls on to the paper which should also be folded and labeled.

For reference purposes in order to obtain a comparative sample from the patient she should be asked to allow about 10 hairs to be pulled from her pubic region.

Step5: Collection of ano-rectal specimens

Conduct a through examination of ano-rectal region in order to record trauma and to obtain biological material for DNA analysis. In cases of possible ano-rectal assault external and rectal swabs should be collected.

The patient should be placed in a comfortable position for an anal examination and swabbing. The swab be slightly moistened with sterile water and the anus carefully swabbed, slightly extending into the anal canal.

Step 5: Genital specimens

Perform a through examination of the genital area in order to identify and record any trauma and to obtain biological material for DNA analysis which will assist in identifying the suspect.

- Swab of the external genitalia in order to collect any saliva or semen that may be present Moisten the swab with sterile water and swab the external and internal surfaces of labia majora, including the clitoris, the periurethral area and the fossa navicularis.
- Take a swab of interior and posterior vaginal fornices using a speculum before an internal digital examination is performed.
- Swab the cervix for collecting as much of mucous plug as possible.

Others:

(1) **Contamination**: Wear gloves at all times both to protect yourself and to avoid contamination of evidence.

Be aware that DNA contamination can occur easily—

Avoid coughing or sneezing over swabs and ensure that any surfaces used for swabs are uncontaminated. Use disposable paper towels on surfaces.

(II) **Reference sample:** Sample provided by a known person, for instance a victim or suspect for DNA analysis are reference samples.

Forensic DNA analysis is a science of comparison and reference blood samples of both victim and suspects are required for comparison of profiles generated from evidence material.

(III) **Swabs and slides for trace evidence:** Sterile swab to be used for the recovery of biological samples from individuals and crime scenes; ensure that these items are guaranteed to be sterile and uncontaminated, with a seal that you can break yourself.

Prepare dry mounts by smearing each swab onto appropriate microscope slide. Allow the smear to air dry, label with examinee's name, date and indicate which swab the slide was made from.

Types of swabs collected for the purpose of a reference DNA samples

Buccal or oral swab (saliva/ bite marks), vaginal and cervix swabs (semen), penial swabs etc.

Label each specimen on the envelopes as follows:

- Name of examine
- Date of examination, time of examination
- Name of individual sample
- Site of collection if applicable
- Name of examining doctor
- Signature of examining doctor on seal of envelops etc.
- FIR No. and Parcel No.
- Description of specimen seal
- Name of the accused/victim

(IV) Fingernail scrapings and trace evidence

Fingernail clippings and fingernail scrapings from the left and right hands should be collected in pre-labeled self-sealing plastic bags and put into labeled envelope.

(V) Hair samples

- Different combs should be used to collect any loose hair or fibres from the head and pubic area over the piece of clean paper. The pubic hair combing and the comb are placed in the envelope.
- Where there is evidence of semen or other matted materials on pubic or head hair, it may be collected with the help of a moistened swab. The swab should be placed in a small paper envelope and labeled "Possible secretion sample from head (pubic) hair.

The second approach is DNA analysis of the hair root and/or sheath of the root. However, the sheath cells surrounding hair roots are more likely to be present when the hair is pulled from the scalp, as might happen, for example during assault/violence.

B. Evidence collection in autopsy samples

Tissue and aborted foetus

- Deep muscle tissue samples and aborted foetus for DNA analysis should be collected in plastic bottles and transported on dry ice.

Bone

- Preferably long bones (femur/humerus) and sternum should be sent for DNA analysis. Totally charred bones should not be sent for DNA profiling.
- Exhumed bones should be cleaned properly. All the sticking debris should be removed.
- Clean bone should be packaged.

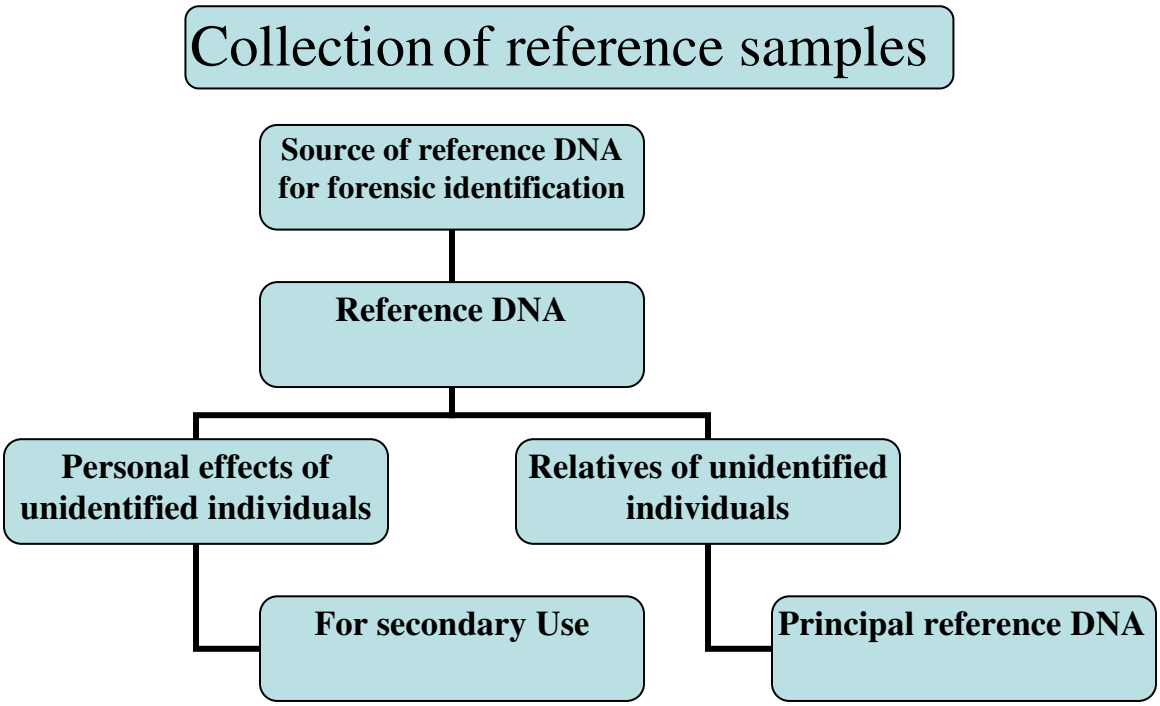
C. Evidence collection from clothing

a. To minimize loss of evidence a hospital sheet should be placed on the floor and a clean paper sheet should be placed on the top of the sheet. The patient should disrobe over the paper sheet.

b. After air drying items such dresses, blouses, shirts etc. should be put into paper bags.

c. Any wet stains, such as blood or semen should be allowed to air dry before being place into paper bags. It is preferable that each piece of clothing be folded inward, placing a piece of clean paper against any stain, so that the stains are not in contact with the bag or other part of the clothing.

D. Evidence collection of reference samples



(I) Conclusive samples from living subjects

- **Blood**

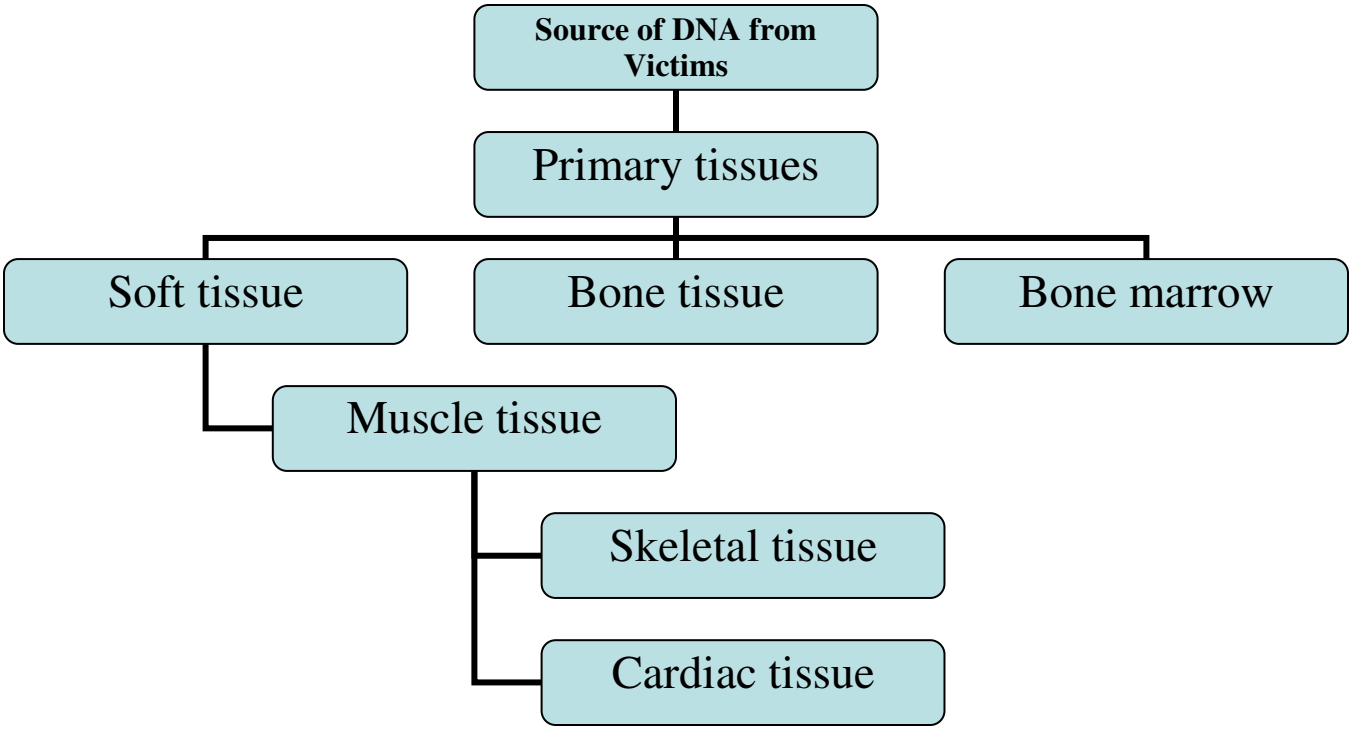
Blood is the classic, conclusive sample for determining DNA. Blood can be obtained by capillary or venous puncture.

- **Buccal epithelial cells**

These cells are collected from the inside of the subject's cheeks, using sterile dry swabs. Two samples are taken- one swab is rubbed on the inside of the left cheek and another swab is used on the right cheek. The swab should be identified and left to dry at room temperature in a protected area. They must not be placed in a container until they are completely dry since the bacteria in saliva proliferate rapidly in moist conditions and will degrade DNA.

- **Hair follicles**

Between 10 and 15 hairs with roots should be pulled from the subject.



(II) Conclusive samples in dead bodies

(a) Conclusive samples in well preserved bodies

- **Post mortem blood.**

  A sample of about 10 ml of blood should be drawn into a tube containing an anticoagulant (EDTA type).

- **Skeletal muscle.**

  Select two skeletal muscle fragments (weighing about 10g and approximately 2 cm wide) from the best preserved area of the body, and place them in a plastic container that has a wide mouth and screw-on lid. This type of tissue is preferable because along with cardiac muscle, it is the most resistant to decomposition.

- **Teeth.**

If there are doubts about the preservation of the corpse, it is advisable to extract four teeth, preferably molars and save them so that exhumation of the body for identification purposes can be avoided. Prior to the extraction, a dental chart should be completed.

(b) Conclusive samples in charred corpses

Despite the external appearance, the stability of DNA at high temperatures allows genetic analysis in corpses where charring is not complete by using fragments of skeletal from deep regions of the body, and from semi-solid blood that remains inside cardiac cavities. If charring is total, it is advisable to contact the laboratory for an evaluation of the available samples and their condition to determine which would be most appropriate for analysis.

(c) Conclusive samples in decomposed or skeletonized corpses

Remaining decomposed tissue should be removed from bone, and a long bone, preferably the femur, should be used. If not possible to obtain this sample, the laboratory should evaluate available samples and their condition to determine which would be most appropriate for analysis.

- **Teeth**

After a dental chart has been completed, select at least four teeth, molars where possible. The samples should not have been damaged or subjected to endodontia.

E. Preventive Guidelines

i. Personnel Protection Guidelines

All body fluids should be regarded as potentially infective.

- Cover any cuts or graze on hands with waterproof dressings.
- Wash hands especially when beginning or ending a new task, before break or meal times, before smoking, and at the beginning and end of duty periods.

ii. Disinfection Guidelines

- Commercial thick bleach can be used for spillages of biologically hazardous materials. This should be left in contact with the contaminated area before rinsing and wiping dry.
- For general disinfection e.g. work surfaces after handling biological specimens, 1 in 10 dilution of commercial thick bleach should be used as above. It should be noted that dilution of thick bleach do not remain effective for periods in excess of a few days.

iii. Taking DNA Reference Samples

- The person taking samples must wear gloves throughout the whole sampling procedure.
- Open the sampling kit and assure that the kit is complete checking off each item against the checklist provided. Follow the sampling instruction.
- If at any time during the sampling process the sample taken is dropped or comes into contact with any other surface the procedure should stop and the sampling kit disposed of. Sample will then be taken using a new DNA sampling kit.
- Once the samples have been successfully taken collect up the wrappers and gloves and dispose of these using designated receptacles.
- Insert the details of the donor and other necessary information on the form provided.
- Place the form together with the sample in the tamper evident container, store and send to the laboratory as per legal instructions.

iv. Anti contamination

- Due to sensitivity of current DNA techniques extreme caution including wearing of a face mask must be taken.
- All containers used for transportation e.g. cool boxes crates, boxes should be cleaned prior to and after use.
- Wherever possible sterile disposables sampling materials should be used.
- Disposable gloves must always be worn over top cuffs and should be changed after handling individual item/objects. Barrier clothing should also be used as often as possible.
- For serious offences wear disposable face masks overshoes and suits fully done up the hood up.

- Handle items as little as possible and not re-open items for interview purposes-use paper bags with transparent panels.
- Always handle one item at a time.
- Where possible take the container to the evidence and not the evidence to the container.
- Contact between victim and suspect samples should be avoided at all times.
- Ensure that any person attending a crime scene has no contact with a suspect or his/her clothing.
- Multiple suspects, the victim and their clothing must be kept apart at all times and should not be allowed to come into contact with the same objects Each item should be packaged sealed and labeled as soon as it is taken.
- Never pack several items/objects together.
- Use bags of a suitable size or shape, do not force items into packaging that are too small, bags may tear or lids may be forced off.
- Seal all packaging securely; use adhesive tape on all edges.
- Never reuse packaging.
- If an item will not fit or packaging is used in error do not use it for a different item. It must be discarded.
- Never eat drink or smoke when recovering evidential samples.
- Dry sample should be kept at room temperature (cool if possible) and out of direct sunlight. Dry sample stored at ambient temperature should not deteriorate/decompose/degrade and will remain suitable for future DNA analysis. Breathable bags, cardboard packaging and brown bags will allow samples to dry out whilst safely packed away and should be stored as above.

- If samples are air dried then this must take place in an area free from any contaminant for example in a sterile drying cabinet. If this is not achievable and there is any risk of minor contamination then samples not be air dried.
- If samples are frozen then they should be kept frozen and never be allowed to thaw and or refreeze since this will cause the break down of DNA.
- Plastic bags can on rare occasions be used to transport very wet items but this should be on the instruction of the local forensic Science Laboratory.
- All sample containing biological materials should be placed into suitable secondary packaging for transport to the laboratory. Local transportation regulations should be adhered to- international biohazard sign can be used.

F. Precautions During Collection and dispatch of samples

(I) Protection of personnel

Prevent, at all times, direct contact by the worker with the sample, using gloves, masks, gowns, or other protective clothing; Prohibit the consumption of food, drink and tobacco products while handling the sample; Maximize asepsis and use disposable materials whenever possible. Once sample collection is complete, places all used disposable materials in containers for biological waste, and follow standards for disposal of biological waste. When sample collection takes place in the autopsy station, extreme precautions should be taken.

(II) Protection of samples

- **Contamination by human biological material.**

This occurs when human biological material is deposited at the site of the event or in the corpse following the event. It can be caused by onlookers, family members, or persons involved in the investigation who accidentally or out of ignorance, contaminate the sample. This occurs frequently when minimal precautions when collecting evidence are overlooked or packaging is defective.

- **Contamination or loss during transfer or biological evidence.**

This occurs, usually accidentally, during the transfer of evidence from one site to another and can result in the contamination or loss of a sample. It happens most frequently when hair samples are moved.

- **Microbiological contamination.**

This type of contamination occurs when microorganisms develop, possibly as a result of humidity or high temperatures. Normally the microorganisms grow or proliferate because of defects in packaging or shortage prior to sending the samples to a laboratory.

- **Chemical contamination.**

This makes it difficult to amplify and extract DNA. It occurs when samples are immersed in preservatives such as formalin or when chemicals have been used in previous tests (for example, fingerprints), thereby compromising DNA analysis.

- **Systems for packing and preserving samples**

Recommended packing and shipping procedures are outlined below:

1. Identification of the samples:

There should be enough space on all of the receptacles to identify the samples and to write the following:

- Reference number of the sample;
- Type of sample:
- Ownership of sample, and location.

2. Chain of custody.

There also should be a space dedicated to the chain of custody with the name and signature of the person who collected the evidence, and the date and hour of collection.

(III) Packaging

(a) Jars or receptacles

With liquid evidence, organs, soft tissue, etc. These receptacles should have screw-on lids or airtight closures; they should already have been sealed with tape and correctly identified, and should be kept refrigerated and sent to the laboratory under refrigeration as soon as possible.

(b) Dry, Sterile swabs.

Swabs used to collect samples will be packed in small cardboard boxes commercially designed for this purpose**.** This type of box protects the swabs and allows them to completely dry out. Once identified. They will be sealed with tape and sent without refrigeration to the laboratory. If it is not possible to obtain specially designed boxes, once the swabs have been used to collect the biological specimen they should be identified and numbered, placed in a protected area, and allowed to dry completely at room temperature before being placed in a shipping container. Once dry the swabs can be placed in correctly identified container, sealed with tape, and sent to be laboratory.

(c) Samples with dry stains.

Each sample is placed on top of paper that will be folded and placed in a paper bag, sealed with tape and correctly identified. This should be sent to the laboratory without refrigeration.

(d) Hairs, nails etc.

This kind of material should be collected in small pieces of paper that will be carefully folded and put in a paper bag, sealed with tape, and correctly identified. This should be sent to the laboratory without refrigeration.

(e) Bones and teeth.

These should be placed in a paper bags and cardboard boxes that are sealed with tape and correctly identified. They can be sent to the laboratory without refrigeration. If tissue is still attached to bones, airtight, plastic receptacles should be sent to the laboratory as soon as possible.

INSTRUCTIONS

- In cases of maternity / paternity dispute blood samples of mother child and suspected biological father are required.
- For identification of deceased/ unidentified corpses/ human remains, blood samples of nearest relatives ie. Father, mother, children, brother, sister are required along with the remains.
- While collecting sample from the dead body, at least two different types of samples are collected – always collect samples in duplicate.
- The samples should be collected in following order of preference-
  - I. 2 to 5 ml blood collected directly from the heart (cardiac puncture)
  - II. In cases where no liquid blood can be obtained skeletal red muscles (50-100 gms) should be collected in DNS.
  - III. Intact long bones in following order of preference-
    - (i) Femur
    - (ii) Tibia
    - (iii) Humerus
    - (iv) Teeth (Preferably Molar)
    - (v) Ribs

IV. In sexual assault cases exhibits collected from the victim/ suspect (garments, swab, slide etc) alongwith blood samples of the victim/suspect and of suspect are required.

SAMPLE COLLECTION KITS/ MATERIAL

(1) EDTA vial for whole blood

(2) FTA classic/ indicating card

(3) Buccal DNA collector

(4) Secur swab