

## Essential Precautions in Sample Collection during Medico Legal Work: A Review

Nishat Ahmed Sheikh\*, Seema S. Sutay\*\*

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### Abstract

Biological materials' collection, preservation and analysis are essential procedures in any Medico legal work. Improper collection of these specimens can greatly alter or negate chemical and toxicological analysis. The investigation of a death from suspected poisoning may depend upon the correctness or otherwise of sampling of fluids and tissues from the body. This article is an update about the standard methods of biological materials collection procedures, preservation and analysis which will be helpful for the Medical and forensic doctors.

**Keywords:** Precaution; Collection; Preservation; Biological materials; Poison; Medico legal cases.

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### Introduction

There are three main sources from which biological material for examination can be obtained, the scene of the crime, the victim and the suspect and his environment.[1,2] Often poison and drugs are involved in medico legal cases where the identification of drugs and poisons is necessary to establish cause of death. In offences involving the human body (assault, rape, murder, etc.) laboratory analysis of material obtained from the victim or the accused can prove vital to proper investigation of the case. Laboratories meant for chemical analysis are located at a distance from the site of occurrence and one has to transport the specimen here for analysis. The prime requisite in transport is to prevent loss of sample and change in chemical and physical attributes of

the specimen so as to avoid misinterpretation of the results.

NCRGSPA (National Committee for Revised Guidelines of Medicolegal Samples Preservation and analysis) has given the following guidelines[1], discretionary power as to whether to have Viscera/body fluids or other biological material analysed in a forensic science laboratory rest with 'Medical Officer' and the Investigating officer. The MO must not collect Viscera/body fluids for analysis as a matter of routine when it is not needed; indulging in such a practice in order to "Buy Time" is unethical and furthermore is indicative of incompetence. Prescribed Forms (Properly filled with relevant details) should be used while forwarding the material. All material should be packed, preserved and sealed properly. Use individualised/departmental/institutional seal for sealing. The covering letter should bear a specimen seal for reference. All material must be packed in suitable containers and each container should have a label affixed to it mentioning relevant details: Nature and quantity of contents, preservative used, name of the deceased, crime no, police station to which the case belongs, PM No, date, name and designation of the medical officer. The exact type of analysis required, should be mentioned in the covering letter. The exact preservative used as well as

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**Authors affiliation:** \*Associate Professor of Forensic Medicine, Kamineni Institute of Medical Sciences, Quarter No: S/III/7, Narketpally, District Nalgonda, Andhra Pradesh, \*\*Associate Professor of Forensic Medicine, RD Gardi Medical College, Surasa, Ujjain, MP.

**Reprints requests:** Dr. Nishat Ahmed Sheikh, Associate Professor of Forensic Medicine, Kamineni Institute of Medical Sciences, Quarter No: S/III/7, Narketpally, District Nalgonda, Andhra Pradesh.

E-mail: drnishatsheikh@gmail.com,

indigofly333@yahoo.com

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**Table 1: Viscera/Body Fluids to be Preserved in All Cases where Toxicological Analysis is Deemed Necessary**

S. No	Material	Quantity
1	Stomach	Entire
2	Stomach content	Up to 300 ml
3	Small Intestine	30 cm (entire length in Infants)
4	Small intestinal contents	Up to 100 ml
5	Liver(portion containing Gall Bladder)	Up to 500 gm (Entire in Infants)
6	Kidneys	One half of each Kidney(Both Kidney in Infant)
7	Blood	10 ml
8	Spleen	½ in Adults (Whole in Infants)
9	Urine	20-30ml.

the quantity should be mentioned in the forwarding letter: e.g. – Sodium citrate 5 mg and 0.1 mg of mercuric chloride mixture for each ml of blood. A sample of the preservative used should also be sent separately.

#### Preservatives

##### Viscera

If specimen can reach the laboratory within 48 hours, there is no need to add preservative to the viscera. Following preservatives used if delay: Alcohol/Rectified Spirit, Saturated solution of common salt, solid common salt. Alcohol is the most suitable preservative, except in poisoning by Alcohol and acetic acid.

*Note:* Never use Formalin as a preservative for sending the viscera to chemical Analysis as it hardens the tissue, renders difficulty to extract poison. In all cases of poisoning, including carbolic acid, saturated solution of common salt should be used as preservative. In cases where poisoning by acids (except carbolic acid), is suspected; rectified spirit should be used as preservative.

Samples for Histopathological examination should be preserved in either 10% formalin / 95 % alcohol. As per dictum the preservative should constitute 10 times the volume of tissue to be fixed.

*For Microbiological Investigation:* Dry ice is best, do not use preservative such as Formalin or Alcohol, Phosphate buffer can be used such that the final pH is between 7.2-7.5, for virological examination: 80 % Glycerol in Buffered saline.

*Blood:* Amount: 10 to 20 ml.

*For Chemical Analysis:* 30 mg of Potassium oxalate + 50 mg of Sodium fluoride for every 10 ml of blood, Sodium fluoride 20 mg for each ml of blood, 10 gm sodium citrate + 200 mg Mercuric chloride in 100ml distilled water. Use 0.5 ml/10 ml of blood.

*For Bacteriology:* 10 ml of whole blood in sterile tube with an Anticoagulant (best Sodium polyanethol sulphate), directly placed into a container of culture medium.

**Table 2: Important Precautions and Guidelines for the Collection of Physical Evidences for Biochemistry**

Sl. No	Sample	Collection
1.	Vitreous Humour	With wide bore needle into syringe, thereafter eyes should be filled with water for cosmetic reasons
2.	CSF	After opening the skull csf should be collected from ventricles

**Table 3: Important Precautions and Guidelines for the Collection of Physical Evidence for Microbiology**

Sl. No	Sample	Collection
1.	Mucous, pus, secretion smears	All smears should be fixed in alcohol before staining
2.	Blood	Preferably blood should be collected from heart with all aseptic precaution and should be sent immediately or should be stored at 40C
3.	Spleen culture	Spleen should be seared with sterile blade
4.	CSF	Through lumbar puncture or cisternal puncture under aseptic precaution

**Table 4: Important Precautions and Guidelines for the Collection of Physical Evidence for Identification**

Samples	Collection Procedures
<b>1) Blood:</b>	
a) Stained clothes	Air dry, Pack each bloodstained garment separately in dry paper parcels. (Pack in dry moisture resistant paper parcels and not in polythene packets).
b) Blood stained immovable articles	Thick Stain: scrap the stained area using a clean knife/blade into a clean dry paper packet. Alternatively swab the area using sterile gauze cloth wetted with normal saline. Each stain should be collected using separate gauze cloth. Air-dry and pack them separately in dry paper parcel. Unused gauze cloth should be sent as control.
c) Soil impregnated with blood	Superficial blood stained soil should be collected and packed in paper parcel. Adjacent unstained soil should also be sent as control.
d) Liquid blood	To be collected using sterile gauze cloth wetted in normal saline. Unused gauze cloth should be sent as control.
<b>2. Semen</b>	
a) Stained clothes	Air dry and packed in dry paper parcels. Avoid folding, crushing the stained area in the process of packing.
b) Swabs, Vaginal swabs	Air dry the swabs and pack in clean dry glass vial/bottle.
c) From immovable articles	To be collected using a sterile gauze cloth wetted in normal saline. Air-dry and pack them separately in dry paper parcels.
d) Pubic hair; Nail clippings	Air-dry and pack them separately in dry glass bottles.
e) Liquid Semen	To be collected using sterile gauze cloth wetted in normal saline. Air dry and pack in dry glass bottle. Unused gauze cloth should be sent as control
<b>3) Saliva:</b>	
a) Liquid saliva	To be collected using sterile gauze cloth wetted in normal saline. Unused gauze cloth should be sent as control.
b) Cigarette butts.	Air-dried and packed in paper parcels.
<b>4) Skin etc.:</b>	
Tissues found at Crime scene	Collected in a clean glass bottle containing saturated solution of sodium chloride.
<b>5) Bone:</b>	Whole bones like Femur, Humerus, rib bones etc., to be collected and packed in dry paper parcels (or) should be kept in aluminium foil and frozen.
<b>6) Teeth:</b>	Teeth to be packed in dry paper parcel.
<b>7) Hair</b>	Hair samples should be collected with rubber tipped forceps air-dried and packed in a paper packet; pack the weapon along with hair sticking to it carefully in paper packet. Preferably 25 control hair samples and as many crime samples.
<b>8) Fibre</b>	Fibre samples should be collected with rubber tipped forceps and packed in a paper packet Tag and place in polythene covers
<b>9) Insects Dead maggots</b>	To be collected in a sterile bottle containing alcohol
Live maggots	To be collected in wide mouthed bottle with a small piece of flesh inside, allow sufficient aeration.
10) Fascia Lata (for chromosomal Studies)	Should be suspended in serum containing antibiotics.

**Table 5: Collection & Storage of DNA Samples**

Sl. No	Sample	Collection
1.	<b>Blood</b>	Should be collected in sterile vial with EDTA 5 ml of blood in case of adults & 2-5ml in case of children.
2.	Blood stained clothes	Blood stained garments should be thoroughly dried in shade & packed in separate paper packet.
3.	Blood stained articles/weapons etc	Scrape the stained area, keep in paper envelope & seal or swab the stains with sterile gauze cloth soaked with saline, dry and pack separately.
4.	Semen/seminal stains/vaginal swabs	Air dry and pack in separate paper packets.
5.	Hairs	Hair with roots to be air dried and packed in separate paper packets.
6.	Tissues of skin, muscle etc.	Collect in a clean glass bottle Add 20% Dimethyl sulphoxide or sodium chloride.
7.	Bones	Whole bones like sternum, femur, humerus to be collected and packed in separate paper packets and kept in aluminium foil and frozen without any preservative.
8.	Teeth	Teeth should be packed in dry paper packets and sealed (Molar or canine are preferred).

*For Grouping:* On filter paper, EDTA bulb (3-5 ml).

*Urine:* Quantity: 20-30ml.

Preservative: 100mg Sodium Fluoride for every 10 ml, Thymol- 0.1gm per 100 ml of urine. Note: For short term preservation refrigeration alone is satisfactory. Collection: Puncturing the bladder with a needle and syringe and aspirate about 20 ml. If less urine, make small incision on the anterior bladder wall, scoop out the urine with a spoon or aspirate it with a syringe or pipette.

*Precaution While Collecting Material for DNA:* Avoid contaminating the area where DNA material is to be preserved, by not touching it with bare hands, or sneezing and coughing over the evidence. Use clean latex gloves for collecting each item of evidence. Gloves should be changed between handling of different items of evidence. Samples should be packed in paper envelopes or paper bags after drying. Plastic bags should be avoided because water condenses in them, especially in areas of high humidity and water can speed the degradation of DNA molecules. Packages should be clearly marked with case number, item number, collection date, and initialled across the package seal in order to maintain a proper chain of custody. Invariably the persons should be directed to FSL for blood collection, but in case they are unable to do so on account of health or age, the sterile vials with preservative may be collected from laboratory. The blood so collected should be labelled and sealed properly and transported in ice so as to reach the laboratory within 24-48 hours. Blood should not be drawn from a person who has undergone blood transfusion till three months. The above precautions and guidelines should be in notice of the medical officer for collection of evidence in an appropriate manner. With every blood sample collected by the medical officer an identification form should be filled up by the individual.

*Preservation in Specific Cases[3,4,5]*

1. In case of burn victims to determine the

levels of carbon monoxide, 10ml of blood sample preserved in liquid paraffin in a glass bottle should be collected and forwarded.

2. Chemical analysis of viscera in case of known cause of death such as electric shock victims and persons known to have died due to diseases like TB, Cancer, Hepatitis, Aids etc. Hence such samples should not be collected for chemical analysis.
3. Similarly it will be of no consequence if chemical analysis of viscera is carried out in case of natural deaths due to starvation, sunstroke, old age, lightning, extreme cold etc.
4. In case of hanging when fracture of hyoid bone concludes death due to hanging, there is no additional advantage in referring the visceral organs for chemical analysis.
5. In case of drowning where the Medical Officer arrives at a definite opinion that the cause of death is due to drowning, no additional purpose will be served by chemical examinations.
6. Testing for Diatoms in visceral organs, spleen & bone marrow may be most useful in cases of drowning. In such case control sample of the water in which body was recovered should be taken in a separate container.
7. In case of snakebite or other insect bites, samples of skin bits of affected area should only be collected and forwarded, with the control samples of opposite site.
8. In cases of hanging, drowning, burns, accidents etc., the Medical Officers may sometimes refer the viscera if any suspicious circumstances arise which are to be noted clearly to conduct proper analysis.
9. In case of deaths due to administering injections the sites of injections, skin subcutaneous tissues along with needle tract weighing about 100 gms should be collected. Similar material from

symmetrical side of the body should also be taken as control in a separate container.

10. In case of inhaled poisons like carbon monoxide, coal gas, hydrocyanic acid, chloroform or other anaesthetic drugs the lung tissues, brain and blood from the cavity of the heart should be preserved and forwarded.
11. Shaft of long bones (8 to 10 cms of femur), a tuft of head hair, finger and torn-nails and some muscles should be preserved in suspected cases of chronic poisoning by heavy metals like arsenic, lead, antimony.
12. In highly putrefied bodies, larvae, maggots, pupa and other entomological samples should be preserved.
13. In embalmed bodies vitreous humour from eyeballs usually remains uncontaminated by the process and may serve the purpose of analysing urea, creatinine and ethyl alcohol.
14. Soil samples from above, beneath and sides of the dead body and control soil samples away from the dead body should be taken in cases of exhumed or skeletonised dead bodies.
15. In case of sexual assault or child abuse, preserve additional samples as required in particular case e.g. swab from bite mark, vaginal wash, breast swab, etc.

#### *Forwarding Samples*

All samples should be properly sealed and labelled with the deceased name, post-mortem number, and nature of sample, collection site, preservative used, date and time of collection. Particular attention should be paid to the packaging of samples to avoid loss during transport, and to comply with health and safety regulations. It should be protected by the use of seals around the lids, and accompanied by an intact chain of custody record. It should be handed over to the investigating officer after obtaining proper receipt.

*Following Documents should be Enclosed along with the Samples[6,7]:*

1. Name, address and phone number of Medical Officer and investigating officer.
2. Circumstances of death and details of drugs thought to be implicated.
3. Past medical history including current or recent prescription medication.
4. Details of emergency hospital treatment and medication given.
5. Copy of forensic pathologist report if available.

#### **Conclusion**

Essential information is stored by proper collection, preservation and analysis of samples obtained in a medicolegal case. Because of discrepancies in procedures at various places, most important evidence may be lost. Hence it is important that standardised procedural guidelines should be implemented, so that the results obtained can be meaningfully interpreted all over India. One must have a keen observation and thorough knowledge related to case keeping in mind all possible outcomes in Forensic Services.

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