METHODS OF COLLECTION, PRESERVATION AND FORWARDING OF BIOLOGICAL MATERIAL FOR DNA FINGERPRINTING

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ABSTRACT

DNA Fingerprinting technique, introduced by Prof. Alec Jeffrey's is widely used presently all over the globe especially for forensic purposes. All living things are composed of different cells and within the cells except red blood cells and few other minor types contain a chemical component called Nucleus. And within the nucleus of each cell is located a genetic material, referred as Deoxyribonucleic Acid (DNA). It is this genetic material which generates genetic pattern of an individual and has shown highly polymorphic among individuals.

INTRODUCTION

It was in 1944, Oswald Avery (1) described De-oxy-ribonucleic Acid (DNA) as the vehicle of generational transference of heritable unit. Later in 1985, Prof. Alec Jeffrey's, while studying myoglobin genes discovered that certain regions of DNA showed variations in the number of tandem repeats. These are known as, Variable Number of Tandem Repeats (VNTR) (2,3). These VNTR's are highly polymorphic and hence are utilized for the forensic purposes.

DNA fingerprinting technique is considered a powerful technique and is widely used all over the globe today. This technique helps in establishing the identity of an individual. It has proved a great help to the justice community. DNA being somatically stable and resistant to environmental degradation has made it a unique tool to be used in forensic medicine (4). Bomjen *et al* (5,6) has shown that DNA can be extracted from biological samples which were stored at various temperatures and for different of interval of time.

Before DNA test is performed on any biological sample, it is mandatory that the sample should be collected, preserved and transported in a proper format for further analysis so as to maintain the chain of custody and moreover to carry out the proper analysis .

COMMON BIOLOGICAL SAMPLES ENCOUNTERED:

- Whole Fresh Blood
- Blood Stain
- Seminal Stain
- Hard Tissues (Bones)
- Soft Tissues (body organs)
- Hair

METHODS OF COLLECTION:

· *Whole blood Sample*: Sterile needle should be used while withdrawing or collecting blood.

· *Blood stain*: Should be picked up preferably on sterile cotton gauge using sterile forceps and blade.

· *Seminal stain*: Should not be touched by hand especially the stain portion. Should be picked up with11

sterile forceps.

· *Hard Tissues*: Bones-- bones should be picked up using gloves, Kept at a place where there are no chances of environmental contamination. It should be allowed to dry completely.

· *Soft Tissues*: Body organs should be collected using forces and wearing gloves. It should be kept in a sterile container.

· *Hair*: Hair roots are preferred for the analysis. Hair roots should be picked up using sterile forceps.

PRESERVATION:

· *Whole Blood*: Blood should be collected in sterile container containing an anticoagulant. The mostly preferred is EDTA. It should be mixed properly but gently for some time. The container should be covered with parafilm to avoid slippage. Should be kept it at 4^0C or using ice during transportation till it reaches laboratory for analysis.

· *Blood stain*: Blood stain should be dried properly. In semi dry stain there, is a possibility of bacterial growth thus chances of having contamination. Drying should be avoided using electric fans. After complete drying it should be wrapped in a fresh blotting paper and packed in a Zip lock poly bag. No preservative is required. It can be transported at environmental temperature.

· *Seminal stain*: Likewise seminal stain should also be dried properly. In semi dry stain there, is a possibility of bacterial growth thus chances of having contamination. It should not be dried using electric fans. After drying it should be wrapped in a fresh blotting paper and packed in a Zip lock poly bag. No preservative is required. It can be transported at environmental temperature.

· *Hard Tissue*: No preservative is required. The hard tissues should be wrapped in the blotting paper and placed in a zip lock poly bag.

· *Soft tissue*: It should be placed at 4^0C or in Ice till it reaches laboratory for analysis.

· *Hair*: Hair roots should be placed in a blotting paper and then packed in a zip lock poly bag. It requires no preservative and can be transported at environmental temperature.

FORWARDING:

· After packing all these zip lock poly bags, they should be numbered and sealed. Each sample, even from the same case should be sealed separately. The seal has to be from the office of the forwarding authority. The covering letter should bear the signature of the forwarding authority and a copy of seal should be enclosed. Wherever photographs are to be attached, the forwarding authority or Doctor collecting blood sample should attest them. While collecting fresh blood, there should be the signatures of minimum two (2) witnesses.

· For forwarding other biological samples also, the signature of minimum two witnesses is required.

REPORTING:

Different labs have different protocols.

· There should be the covering letter, either from HOD or Head of the Institute mentioning the name of the person who has carried out the analysis. Also, it should be mentioned who should be called for disposing evidence in the court.

· The report should mention the exhibits examined with the number given by the forwarding authority or the one decoded by the laboratory

· In case of matching, report should indicate what are the points considered for it, by marking arrows.

· It should be evident that the bands in the child are those determined from each parent.

· Preferably, the molecular weight marker should be run along with DNA extracted from the biological samples.

· The name of the probe (s) or primers should be mentioned.

· The DNA sample of an unrelated individual should also be run along with the DNA samples extracted, pertaining to a particular case.

· A negative control should also be run so as to check the contamination or unspecified product.

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References:

1. Avery, O.T., Macleod, C.M., and McCarty,M (1994)Studies on the chemical nature of the substance inducing transformation of Pneumococcal types. *J.Exp.Med*., ;79:137-158
2. Jeffreys, A.J., Wilson, V., & Thein, S.L (1985a) Hypervariable “minisatellite” regions in human DNA. *Nature;*314: 67-73
3. Jeffreys, A.J., Wilson, V., & Thein, S.L (1985b) Individual specific “fingerprints” of human DNA *Nature* ;316: 76-79
4. Kirby Lorne T (1990) DNA Fingerprinting:AN introduction, M Stockkton press.
5. Ginya Bomjen, Anupuma Raina, Irshad M Sulaiman, Seyed E Hasnain & TD Dogra (1996) Effect of storage of blood samples on DNA yield, quality and fingerprinting: A forensic approach. *Indian Journal of Experimental Biology*;34:384-386
6. Bomjen G, Raina A, Sulaiman I M, Hasnain S E & Dogra TD (1994) Effects of various storage of human tissues in DNA fingerprinting.J Forensic Med & Toxicology;3:1-6