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Touch DNA: An Important Clue in Criminal Cases

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Abstract

Advanced DNA technology, such as Polymerase Chain Reaction (PCR), makes it possible to obtain conclusive results in cases where previous testing might have been inconclusive. Polymerase chain reaction (PCR) based Short Tandem Repeats (STR) Technique becomes an important tool to solve various types of criminal cases. In 1910 Edmund Locard established the first crime laboratory as a professor of forensic medicine at University of Lyons, France and is best known for his evidence transfer theory in forensic science. According to Locard's exchange principle hold that the perpetrator of a crime will bring something into the crime scene and leave with something from it, and both can be used as forensic evidence that means "Every contact leaves a trace." Wherever accused commits any crime, whatever he touches, whatever he leaves, even unconsciously, will serve as a silent witness against him/her. Mainly in rape-cum burnt suicidal cases improper swab samples sent by medical officer is becomes less exclusive and such silent evidence becomes challenging to find nature of crime and real culprits. DNA can be obtained from traces of biological fluids on clothing exhibits. But sometimes due to limitations in detection we can't find out the stains of ideal biological fluids (blood, semen etc) on such cloth articles. But on the other hand, forensic expert's interest to study the case in different way. Other than ideal fluids sometimes sweat stain detected on cloths will be helpful to establish the identity of real culprits. Mix DNA profile obtained from sweat stain on cloth articles of accused and deceased becomes a challenging job to prove involvement of the criminals. DNA sequence cannot easily be changed once the material is left at a crime scene, thus increasing its effective use in forensics, and the probability of finding an exact match. Today DNA profiling took huge strides forward in both discriminating power and the ability to recover information from any type of exhibits by extracting DNA from sweat stains on cloths also.
"Touch DNA" help to solve mysterious case rape with murder and destruction of evidences by burning.

Keywords
Sweat stain, Touch DNA, Polymerase Chain Reaction, STR, DNA profiling

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INTRODUCTION:

Humans sheds tens thousands of skin cells each day, and these cells may be transferred to every surface our skin contacts [1]. When a crime is committed, if the perpetrator deposits a sufficient number of skin cells on an item at the scene, and that item is collected as possible evidence, touch DNA analysis may be able to link the perpetrator to the crime scene. Touch DNA is also called as Contact Trace DNA. It refers to the DNA that is recovered from skin

(epithelial) cells that is left behind when a person touches or comes in contact with items such as clothes, a weapon or other objects [2]. DNA profiling from swabs of palm and touched items like handles, pens, phones etc was first demonstrated in 1997[3]. Increasingly touched evidence materials useful for crime investigation are subjected to genetic profiling. Touch DNA doesn't require us to see anything, or any blood or semen at all. It only requires seven or eight cells from the outermost layer of our skin. Touch DNA has been successfully sampled from countless items including mobile phones, gun grips, steering wheels, eating utensils, and luggage handles, just to name a few[4,5].However, since Touch DNA is usually deposited in smaller amounts than the DNA found in bloodstains or other body fluids, it is more difficult to obtain DNA profiles from touch DNA samples. The key to obtaining successful Touch DNA results depends on recognizing items which may be suitable for Touch DNA analysis and using the sampling technique that will recover the highest number of skin cells.

Cellular material, and in particular DNA, is transferred to fabrics when a person makes direct contact. It is assumed that the amount of cellular material and associated DNA that will be transferred increases with increasing time and pressure applied to the garment. In such transfer events there is no indication of where on the fabric the cellular material may have been deposited, unless there is distortion to the fabric.[6] Low levels of DNA recovered from touched items can be close to the limit of detection for current STR-based DNA profiling kits. The success of a DNA profile depends largely on how much DNA is retrieved from the evidence [7].

Sweat contains extracellular DNA that might contribute to the DNA profiles obtained from touched surfaces [8,9]. The latter has recently been proven by Quinones and Daniel who detected cell free DNA in 80% of the healthy individuals whose sweat was analyzed [10]. The genetic material to be fingerprinted can come from a variety of sources including: blood, saliva (containing epithelial cheek cells), and semen or other less likely samples such as skin, bone, teeth or hair .But one of the most important stain of sweat also becomes important in some cases where there is no any traces of ideal biological fluids remains at scene of crime [11].

DNA Fingerprinting in recent years has become more widely used by police in areas of violent crime and in solving Rape, Murder and Paternity and disputes cases. There is no doubt that DNA is a valuable resource to forensic science, but we must consider how this technology actually works, and if it is accurate and practical enough to have widespread implications on the legal system. The techniques used today are either based on the type of genes an individual has (dominant or recessive) or on the amount of reoccurrence of a certain pattern of nucleotides in the genetic material. In the case of the polymerase chain reaction technique, the gene types of an individual are matched to obtain a DNA fingerprint, while in the case of Restriction Fragment Length Protocol (RFLP), VNTRs and STR methods the repetition of nucleotide patterns within a chromosome are measured to obtain a fingerprint [11, 12].

In the course of a criminal investigation, DNA is often recovered from items that have been handled by an individual. Whilst there have been studies investigating the propensity of different individuals to deposit DNA, little is known about the factors involved in the transference of DNA through touch. This investigation seeks to clarify some of the underlying processes involved in DNA transfer, as to better understand the significance of so-called ''touch DNA'' evidence (t DNA) [13]. Among possible substrates, clothing is often thought to be a source of important evidence for forensic DNA analysis in criminal investigations, e.g. In sexual assault cases [14]. It is known that touch DNA can be recovered from the bodies and clothing of rape victims, but useful profiles are difficult to acquire since touch DNA is usually deposited in small amounts compared to bloodstains or other bodily fluids [15]

MATERIALS AND METHODS:

Materials:

Table 1 Reagents and Chemicals

Reagents	Parameters
Forensic Buffer	1 ml Tris HCL-100ml 0.5ml EDTA Buffer -10ml 5M Nacl-10ml Make the volume up to 1000ml
Proteinase K	Appearance- Colorless solution in 50% glycerol, cont.20mM Tris.,1mM $CaCl_2$, PH ca.7.4 Concentration 20mg solid/ml
Investigator kit	Buffer G2, Prot. K, Carrier RNA,
Amp FSTR Identifier® PCR amplification Kit	Allelic Ladder, Ampli Taq Gold® DNA polymerase, Primers,
Hi-Di™ Formamide	CAS 75-12-7, CAS 60-00-4
Size Standard	GeneScan™-500, LIZ ™

Table-2 EZ1 Automate DNA Extraction System Parameters

Instrument	Operating Parameters
Kits designed for this instrument	QIAGEN EZ1 Kits
Pipeting range	50-1000 µl
Protocols/main application on this instrument	Purification of DNA, mRNA, total RNA, and viral RNA and DNA
Samples per run; throughput	6 samples per run
Technical data of the instrument	Weight 48 kg, 100–240 V AC, 50–60 Hz
Technology	Magnetic-particle technology

Table-3 Polymerase Chain Reaction Thermal Cycler Machine

Instrument	Operating Parameters
Capacity	96 wellx0.2ml PCR tubes/one 96 well plate
Heating/cooling	Peltier based
Capable of testing temperatures	Denaturation, Annealing & Extension steps
Block ramp rate	5.0°C/Sec.
Sample ramp rate	4.4°C/S
Temperature range	4-99°C/S
Temperature accuracy	±0.2°C
Customized programming	Allows a maximum of 20 steps and 99 cycles
Display	LCD touch screen, about 8.5 in

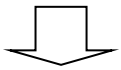
Table-4 Genetic Analyser-3130

Instrument	Operating Parameters
Fragment Size(bp)	500bp
No. of Markers	16
Polymer	POP4
Detector	CCD
Oven Temp	60 °C
Column Size	36cm
Software	Gene Mapper®

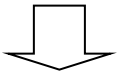
Methods:

Protocol-1: Extraction of DNA from sweat stains and nails

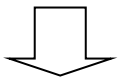
Carefully clean the platform of workstation of laminar flow with ethyl alcohol



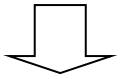
Take approx 0.5mm sample piece and cut respective sample into small pieces and take into 2ml micro-centrifuge sample tube



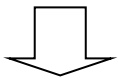
To this Add 400µl Forensic Buffer +25 µl Protease K+40 µl 1mM DTT



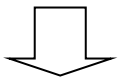
Vertex and spin



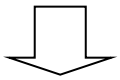
Incubate at 56˚C overnight on Thermo-Shaker



To the next day, transfer the sample into the EZ1 micro-centrifuge sample tube



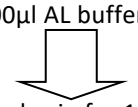
Set the micro-centrifuge sample tube in the EZ1 Advanced (Quiagen) magnetic bead based liquid handling system for automate DNA Isolation



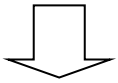
Store the extracted DNA at -20˚C

Protocol-2Extraction of DNA from Blood

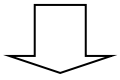
Take 5 µl blood sample+ 97.5 µl ATL buffer+100µl AL buffer+10 µl protease K in micro-centrifuge Tube



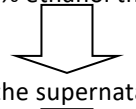
Vortex and spin for 1 min's



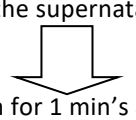
Incubate at 56˚c for 10 min's on Thermo-shaker



To this add 50 µl 99% ethanol then vortex and spin



Wait for 5 min's and transfer the supernatant into the micro-kit column



Centrifuge at 80000rpm for 1 min's and discard the filtrate

Wash the column by adding 500 µl washing solution

Centrifuge the column at 8000rpm for 2min's and discard the filtrate

Repeat the procedure

Spin the empty column at 10000rpm for 3min's

Transfer the column in new Elution Tube

Finally add 100 µl Elution Buffer to the column, centrifuge at 14000rpm for 2 min's

Remove the column and store the DNA for at -20˚C

Polymerase chain reaction (PCR)
Reagents Volume:
PCR reaction Mix -10.5 ul
Primer set- 5.25 ul
Sample input -10 ul
Protocol:

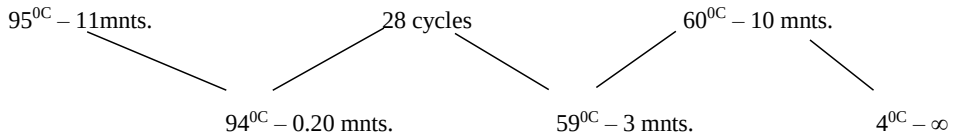
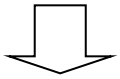


Fig.1

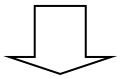
Genotyping
STR genotyping is detected and analysed on 3130 Genetic Analyser (Applied Biosystems) instrument by capillary electrophoresis of single stranded amplified DNA fragments includes following steps.
Sample Preparation for Injection
Standard Mix:
1µL Single or pooled PCR product
0.5 µL Size standard (GeneScan500-LIZ®only 0.3 µL)
10-20 µL Hi-Di™ formamide (PN 4311320)

Protocol:

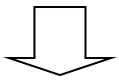
Denaturation of PCR product.
(90 –95 °C, 2 –5 min)



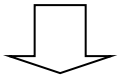
Immediately on ice or cool to 4 °C in thermal cycler
Load the mixture in auto sampler on instrument for injection.



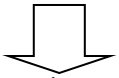
Electrophoresis is done through fine glass capillary filled with polymeric gel.
(During capillary electrophoresis, the extension products of the PCR reaction (and any other negatively charged molecules such as salt or unincorporated primers and nucleotides) enter the capillary as a result of electro kinetic injection. The extension products are separated by size based on their total charge)



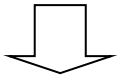
DNA fragments travel through capillary according to their size & reach the window which Coincides with the Laser device in the instrument. (Shortly before reaching the positive electrode, the fluorescently labeled DNA fragments, separated by size, move across the path of a laser beam. The laser beam causes the dyes attached to the fragments to fluoresce.)



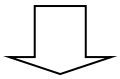
Laser excites the fluorescently labeled DNA fragments.
(The laser beam causes the dyes attached to the fragments to fluoresce.)



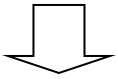
CCD Camera behind the window records the excitation peaks.
(The dye signals are separated by a diffraction system, and a CCD camera detects the fluorescence.)



Excitation peaks for 16 different loci are obtained.
(Because each dye emits light at a different wavelength when excited by the laser, all colors, and therefore loci, can be detected and distinguished in one capillary injection.)



For each set of sample standard allelic ladder is run.



DATA COLLECTION software collects the data of these excitation peaks.
(The fluorescence signal is converted into digital data, and then the data is stored in a file format compatible with an analysis software application.)

RESULTS AND DISCUSSION:

The DNA was extracted from sweat stain from partly burnt cloth piece of brassier of victim/deceased and blood sample, nail of accused, sweat stain from sandow baniyan and nicker of accused was typed at 15 STR LOCI and gender specific Amelogenin locus using PCR Amplification technique.

Table 4-The results of DNA typing is summarized below:

STR LOCUS	GENOTYPE			
	DNA profile of Partly burnt cloth piece of brassier of deceased	Mixed DNA profile of Sandow baniyan of accused	DNA profile of Blood and nail of accused	DNA profile of Nicker of accused
D8S1179	10,14	9,10,14,15	9,15	9,15
D21S11	30,31.2	30,31.2, 32,32.2	32,32.2	32,32.2
D7S820	10,11	8,10,11	8,11	8,11
CSF1PO	12,13	11,12,13	11,13	11,13
D3S1358	14,18	14, 17,18	17,18	17,18
THO1	6,9	6,9	6,9	6,9
D13S317	9,12	9, 11,12	11,12	11,12
D16S539	11,12	9,11,12	9,12	9,12
D2S1338	19,23	18,19, 21,23	18,21	18,21
D19S433	13,14	12,13,14,16	12,16	12,16
vWA	17,17	16,17,18	16,18	16,18
TPOX	8,11	8,11,12	8,12	8,12
D18S51	13,14	13,14	13,14	13,14
AMELOGENIN	XX	XY	XY	XY
D5S818	12,13	11,12,13	11,12	11,12
FGA	20,22	20,22,23	20,23	20,23

In table 4 it is clearly shown that**,** mixed DNA profile obtained from sweat stain detected on baniyan of accused

a) One of DNA profile **matched** with the control DNA profile obtained from sweat stain detected on partly burnt cloth piece of brassier of deceased.
b) Other DNA profiles **matched** with the control DNA profile obtained from of blood sample, nail and sweat stain detected on nicker of accused.

i.e. mixed DNA profile is **identical and from one and same source of female (deceased) as well as male(accused) origin**.

Now a day's rate of rape cases is rapidly increasing. Due to the psycho mind of criminals many life's were got spoiled. Today it becomes very difficult for the police to make a judgment of the nature of crime, punishment to criminals and give justice to victim, due to unavailability of strong evidence in the judicial process.

Forensic science is the art in order to determine how the crime was committed and involvement who is real culprits. DNA evidence is one of the most prominent pieces of evidence that is used in the judicial system today. In the past when someone committed a crime such as a sexual assault, unless there were witnesses there was no real way of proving that a specific person was guilty. In rape cum bunt cases it becomes very difficult to medical officer to do the proper sampling and forensic expert to analysis of such samples of victim/deceased as well as the detection of any biological fluid traces on burnt cloth pieces of victim/deceased. Hence it becomes less exclusive in DNA analysis. But on the other hand; in the interest of biological fluids (other than blood and semen) like sweat helps to solve such type of challenging cases and elevate the level of justice through DNA-Fingerprinting. DNA forensics, a level of certainty can be established that is recognized as valid evidence in a criminal case, either for the prosecution or the defense. Several years later the fingerprinted evidence will appear on the witness stand exhibited by a forensic scientist.

ACKNOWLEDGEMENT

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REFERENCES:

1) Lowe A, Murray C, Whitaker J, Tully G, Gill P. The propensity of individuals to deposit DNA and secondary transfer of low-level DNA from individuals to inert surfaces. Forensic Sci Int 2002;129(1):25e34.
2) Bright, J and Petricevic SF. Recovery of trace DNA and its application to DNA profiling of shoe insoles. Forensic Sci. Int. 2004; 145: 712
3) Van Oorschot RAH, Jones MK. DNA fingerprints from fingerprints. Nature 1997; 387:767.
4) Wickenheiser RA. Trace DNA: a review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact. J Forensic Sci 2002;47(3):442e50.
5) B. Budowle, et al., Simple protocols for typing forensic biological evidence: chemiluminescent detection for human DNA quantitation and restriction fragment length polymorphism (RELP) analyses and manual typing of polymerase chain reaction (PCR) amplified polymorphisms, Electrophoresis 16 (1) (1995) 1559–1567.
6) R.A.H. van Oorschot, D.G. Phelan, S. Furlong, G.M. Scarfo, N.L. Holding, M.J. Cummins, are you collecting all the available DNA from touched objects, International Congress Series 1239 (2003) 803–807.
7) Jennifer Templeton a, *, Rene´e Ottens a, Viviana Paradiso a , Oliva Handt b , Duncan Taylor b , Adrian Linacre b Genetic profiling from challenging samples: Direct PCR of touch DNA fragmented DNA on touched objects, Forensic Sci. Int. Genet. 3 (2008) 32–36.
1. A. Linacre, V. Pekarek, Y.C. Swaran, S.S. Tobe,Generation of DNA profiles from fabrics without DNA extraction, Forensic Sci. Int. Genet. 4 (2010) 137–141.
2. I. Quinones, B. Daniel, Cell free DNA as a component of forensic evidence recovered from touched surfaces, Forensic Sci. Int. Genet. 6 (2012) 26–30.
3. A.J. Jeffrey's V.Wilson, R.Neumann and J.Keyte " Amplification of Human Minisatelites by the polymerase chain reaction towards DNA Fingerprinting of single cell" Nucl.Acids Res. 16 (1988) 10953 -71
4. R.Reynolds, G.Sensabaugh, and E. Blake "Analysis of Genetic markers in forensic DNA samples using the polymerase chain reaction" analytical Chemistry 65 (1991) 2 – 15.
5. Scientific working Group on DNA Analysis Method (SWGDAM) (200) short tandem repeats (STR) interpretation guidelines. Forensic Science Communication 2(3) Available online at: http://www.fbl.gov/hq/lab/fsc/backissu/july2000/string.htm.
6. Identification of Criminal by using Touch DNA: A new Tool for Investigation in Forensic Science Mahipal Singh Sankhla & Dr. Rajeev Kumar Student of M.Sc. Forensic Science, Division of Forensic Science, Galgotias University Greater Noida. Assistant Professor, Division of Forensic Science, Galgotias University Greater Noida.
7. Schulz MM, Reichert W (2000) A strategy for STR-analysis of cryptic epithelial cells on several textiles in practical casework. In: Sensabaugh GF, Lincoln PJ, Olaisen B (eds.) Progress in forensic genetics. Elsevier: Amsterdam, The Netherlands 8: 514-516.
8. Gill P (2001) Amplification of low copy number DNA profiling. Croat Med J 42: 229-232