CTT Multiplex System is a quick and inexpensive method to exclude innocent suspects during criminal inquiries

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Abstract. CTT multiplex (CSF1PO, TPOX, THO1) is a highly polymorphic STR among most populations. During criminal inquiries there is a great need for a rapid and inexpensive method to exclude innocent suspects and to zoom down further inquiries on the smallest possible number of suspects. In this article we present 5 legal cases representing different applications of CTT print. Those applications included inexpensive search for the true offender among large number of suspects, solving human identification problems in cases where tiny amounts of biological material is available such as cigarette butts or residual bone tissue resulting from sawing a corpse, establishing a crime of committing adultery on a married woman by exclusion of biological paternity of her child to her husband and quick liberation of those innocent suspects in custody if CTT print excluded their presence at the crime scene. The results presented herein suggest that CTT multiplex serves as a DNA fingerprint in cases where discordance of DNA prints are sufficient to make a decision. [Nature and Science. 2008;6(2):6-19]. ISSN: 1545-0740.

Keywords: CTT Multiplex; Exclusion; Forensic; Inexpensive; Suspects

INTRODUCTION

DNA fingerprinting is a very important tool in the search for justice as it provides prosecutors with a way to pinpoint suspects with a high degree of certainty, and on the other side can exonerate others without the expense and suffering caused by a trial (Clayton *et al*., 2004).The amplification of short tandem repeat (STR) loci using the polymerase chain reaction (PCR) is currently the method of choice in all forensic investigations. Sprecher *et al*., 1996 having several advantages over conventional Southern blotting method of the larger variable number of tandem repeats (VNTRs) (Kirby and T. Lorne,1990). producing reliable and highly discriminating results with 1ng or less of sample material where casework samples (ex: blood stains, hair roots, cigarette buts, seminal stains,…etc) may contain limited amounts or partially degraded DNA as the only evidence that may link a suspect to a crime (Edwards, 1991**)**. Discrete alleles from STR systems may be obtained due to their smaller size, which puts them in the size range where DNA fragments differing by a single tri or tetra-nucleotide repeat in size may be differentiated. Determination of discrete alleles allows results to be compared easily between laboratories without binning. The inclusion of allelic ladders with each STR multiplex system provides a rapid and accurate method of allele determination that is easy to present to a judge without necessitating a detailed explanation of the underlying scientific principles (Egyed *et al*.,2006)

CTT marker (table1) is one of the most polymorphic multiplex systems characterized by high heterozygosity, distinguishable alleles, regular repeat unit and robust amplification

Table (1): Locus-Specific Information (Edwards, 1991).

STR locus	Chromosomal location	Locus definition	Repeat sequence 5' - 3'
THO1	**11**p15.5	Intron 1 of human tyrosine hydroxylase gene	$AATG^2$
TPOX	**2**p25.1-pter	Intron 10 of human thyroid peroxidase gene	$AATG^2$
CSF1PO	**5**q33.3-34	Human c-fms proto-oncogene for CFS-1 receptor gene	$AGAT^2$

In police investigations CTT marker serves as a rapid DNA profiling test which provides a powerful scientific tool that helps detecting the criminal who usually confesses before being presented to court. Hanson and Ballantyne, 2005. However in those cases where several persons are suspected and police investigators need to free those innocent suspects whose DNA prints are not related to biological remains left at crime scene, a quick inexpensive test is required for rapid decision making. In this manuscript, the significance of using the CTT multiplex to exclude innocent suspects is clarified in various forensic applications (murder, robbery, rape disputed paternity, and identification of dead body remains). Five legal cases are presented where different biological impacts were left at crime scenes (ex: dried blood stains, hairs, cigarette buts, muscle tissues, seminal fluid and vaginal secretion).

MATERIALS AND METHODS

DNA Extraction:

Extraction of total human genomic DNA was performed on biological samples left at crime scenes using 5 % Chelex 100 as a medium for extraction according to the following protocols:

1. a. Blood samples: 150 µl of whole blood, or 1 cm^2 portion of blood stained material was placed into a sterile 1.5 ml microcentrifuge tube containing 0.4 ml of sterile deionized water, then mixed gently and incubated at room temperature for 15 minutes, Centrifuged at 13,000 rpm for 5 minutes, then Carefully all but 20-30 µl of the supernatant was removed from each sample and discarded leaving the remaining pellet containing DNA.
   b. Hair samples: Samples were placed into a 1.5 ml mirocentrifuge tube containing sterile deionized water and shaken vigorously to remove surface dirt and to reduce contamination.
   c. Cigarrate buts: samples were prepared by cutting a 0.5 cm wide strip from the filter end of each cigarette butt, then the strip was cut into smaller pieces and placed into a sterile 1.5 ml microcentrifuge tube.
   d. Seminal stains, vaginal stains and buccal swabs: 1 cm^2 portion from the sample was placed into a sterile 1.5 ml microcentrifuge tube containing 0.4 ml of sterile deionized water, then mixed gently and incubated at room temperature for 15 minutes, centrifuged at 13,000 rpm for 5 minutes, then carefully all but 20-30 µl of the supernatant was removed from each sample and discarded leaving the remaining pellet containing
2. (30-100) µl of 5 % Chelex 100 were added according to the type and volume of the sample.
3. (3-10) µl of Proteinase K (10 mg / ml) were added according to the type and volume of the sample .
4. 10 µl of 1M Dithiothreitol (DTT) were added. (On seminal, vaginal, and buccal swab samples only).
5. Samples were incubated at 56 °C for (30min.-overnight) according to the type of the sample then vortexed on high speed for 5-10 seconds.
6. Samples were incubated at 100 °C for 8 minutes, vortexed on high speed for 5-10 seconds, and then centrifuged at 13,000 rpm for 5 minutes at room temperature. Finally the DNA, which is in the supernatant, is ready for amplification.

DNA Amplification:

For each sample, DNA amplification was performed in a sterile laminar flow hood. The reaction mix contained the following components

PCR Master Mix Component	Volume Per Sample (µl)
Sterile deionized water	14.8
STR10x Buffer	2.5
CTT Multiplex 10x Primer Pair Mix	2.5
Taq DNA polymerase (at 5 u / µl)	0.2
Extracted DNA sample	5
Total volume	25

Amplification was performed according to the following thermal cycling protocol

Initial incubation	Cycling for first 10 cycles	Cycling for last 20 cycles	Hold step
96 °C for 2 minutes	94 °C, 1 minute 64 °C, 1 minute 70 °C, 1.5 minutes	90 °C, 1 minute 64 °C, 1 minute 70 °C, 1.5 minutes	4 °C

A positive control was included by adding 5 µl of K562 DNA instead of sample DNA to 20 µl of PCR master mix. A negative control was included by substituting sample DNA with 5 µl of sterile deionized water in a tube containing 20 µl of PCR master mix. At the end of thermal cycling samples were stored at -20 °C until performing polyacrylamide gel electrophoresis.

Polyacrylamide gel electrophoresis

Amplified DNA samples were prepared by mixing 1 to 1 (V/V) with 2x STR loading solution containing (10mM NaOH, 95% formamide, 0.05% bromophenol blue and 0.05% xylene cyanol FF) followed by heating at 95°C for 3 minutes then chilling immediately in ice. The polyacrylamide gel was pre-run at 40 watts for at least 30 minutes. Denatured samples were resolved on 4% polyacrylamide gel in 0.5 X TBE buffer using a sequencing gel apparatus (30 cm width x 40 cm length). Samples were allowed to resolve at 40 watts for at least 75 minutes.

Silver Nitrate staining

After electrophoresis, plates were separated carefully using a plastic wedge. The gel (attached to one plate) was placed in a shallow plastic tray, and then subjected to treatment with silver stain according to the following steps:

Step	Solution	Times
a	Fix / stop solution	20 minutes
b	Deionized water	2 minutes
c	Repeat step b, twice	2*2 minutes
d	Staining solution	30 minutes
e	Deionized water	10 seconds
f	Developer solution	Up to 5 minutes (until alleles and ladders are visible)
g	Fix / stop solution	5 minutes
h	Deionized water	2 minutes

The gel (on a plate) was positioned upright and allowed to dry then photographed for documentation.

RESULTS

CASE NO 1

Crime scenario

A master felon committed more than 15 crimes including robbery and setting fire, and never leaves behind him any materialistic evidence that indicates his presence in any of the places where he committed his crimes. There was only one evidence that indicates his responsibility for the crime; a paper he usually writes while planning for each crime. He leaves this paper for the investigators at the crime scene as a kind of belittling the policemen abilities in solving the mystery of the crime as well as threatening them to commit other similar crimes. One night, this felon decided to rob a safe in the office of the undersecretary of the ministry of health and population located in the training center building in Asyout (a major city in Upper Egypt). At 2:00 am, the felon sneaked into the office, turned over the safe, and started to saw certain part of it using some metal tools that he had brought. While he was sawing the safe, his forefinger was

injured and a drop of blood fell on the metal body of the safe front from the inside. The criminal lab expert removed the blood stain using a piece of gauze wetted with de-ionized water and sent it to the central criminal lab for analysis. Results were documented for future comparisons. Because of the high proficiency that was noticed in all those crimes, it was decided to inspect CTT print in all felons having similar criminal records. Besides, the CTT print of the blood stain was compared with prints stored for police staff databases. Surprisingly an ex-sergeant who left the service after several years working with the criminal inquiry staff had a similar CTT print to that found at crime scene.

DNA Analysis

A sample DNA profiling gel showing CTT multiplex of 4 suspects plus the offender and the blood stain CTT multiplex.

Analysis was carried out using polyacrylamide gel electrophoresis followed by silver stain detection and the results were shown in fig (1).

The lanes contain the following samples:

- Lane 1: CTT allelic ladder.
- Lane 2: -ve control.
- Lane 3: +ve control.
- Lane 4: Blood stain at the crime scene (over the safe).
- Lane 5: Blood sample taken from the ex-police staff.
- Lane 6: Blood sample taken from felon 1
- Lane 7: Blood sample taken from felon 2.
- Lane 8: CTT allelic ladder.
- Lane 9: Blood sample taken from felon 3.

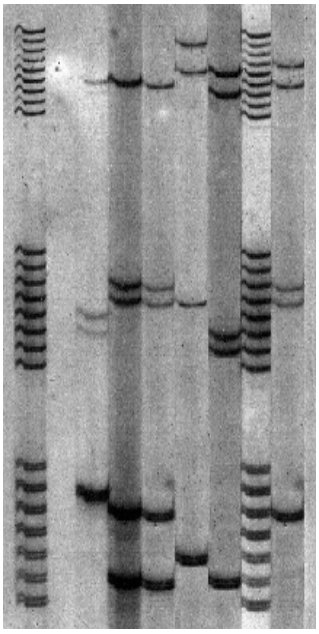

Fig (1): Case no 1

Interpretation of results

CTT profile of the sample in lane no 4 is similar to the CTT profile of the sample in lane no 5 as both have the profile 6-9, 10-11, 10-10 for the genetic loci: THO1, TPOX and CSF1PO respectively. None of the other suspects had similar CTT print to that of the blood stain.

Trial

Investigation authorities decide that the second, third, and fourth suspects are innocent and immediately set them free. Both ex-sergeant's blood and blood stain were further analyzed by CODIS STR loci to create a compelling evidence on the true offender before sending him to court.

Case no 2

Crime scenario

A criminal decided to kill his rival who swindled him out of a great sum of money. He visited him in his apartment where they had supper and smoked cigarettes then the felon volunteered to prepare tea. He went to the kitchen, brought a big knife, sneaked to his victim, and stabbed him in his back. The victim tried to defend himself, holding the felon's head but in vain, the criminal stabbed him a number of times in the chest. After making sure of his victim's death, the criminal took the knife and escaped.

When the investigators entered the apartment they found the corpse of the victim in the midst of a blood pool in the hall. Entertainment impacts and two cigarette buts were found on the table beside the corpse. The criminal lab expert took a sample of blood from the knife wound in the chest of the corpse. While examining the corpse, he noticed that the victim's right hand fingers are contracted gripping some locks of hair (snatched from the head as they have their roots). He took these snatched locks and the two cigarette buts found on the table beside the corpse for analysis. A week later, the criminal inquiry resulted in restricting the suspicion to four persons, as there was a great hostility between these persons and the victim on materialistic affairs.

DNA Analysis

Whole blood samples were taken from those four suspects and analyzed in comparison to the samples taken from the crime scene.

DNA profiling test was carried out on the crime scene samples and the samples taken from the four accused persons simultaneously using the CTT multiplex. Analysis was carried out using polyacrylamide gel electrophoresis followed by silver staining and the results are shown in figure (2).
The lanes contained the following samples:

- Lane 1: blood sample taken from a knife wound from the chest of the corpse.
- Lane 2: CTT allelic ladder.
- Lane 3: blood sample taken from the blood pool surrounding the corpse.
- Lane 4: one cigarette butt found on the table beside the corpse.
- Lane 5: another cigarette but found on the table beside the corpse.
- Lane 6: hair lock found in between the right hand fingers of the victim's corpse.
- Lane 7: blood sample taken from suspect 1.
- Lane 8: blood sample taken from suspect 2.
- Lane 9: blood sample taken from suspect 3.
- Lane 10: CTT allelic ladder.
- Lane 11: blood sample taken from suspect 4

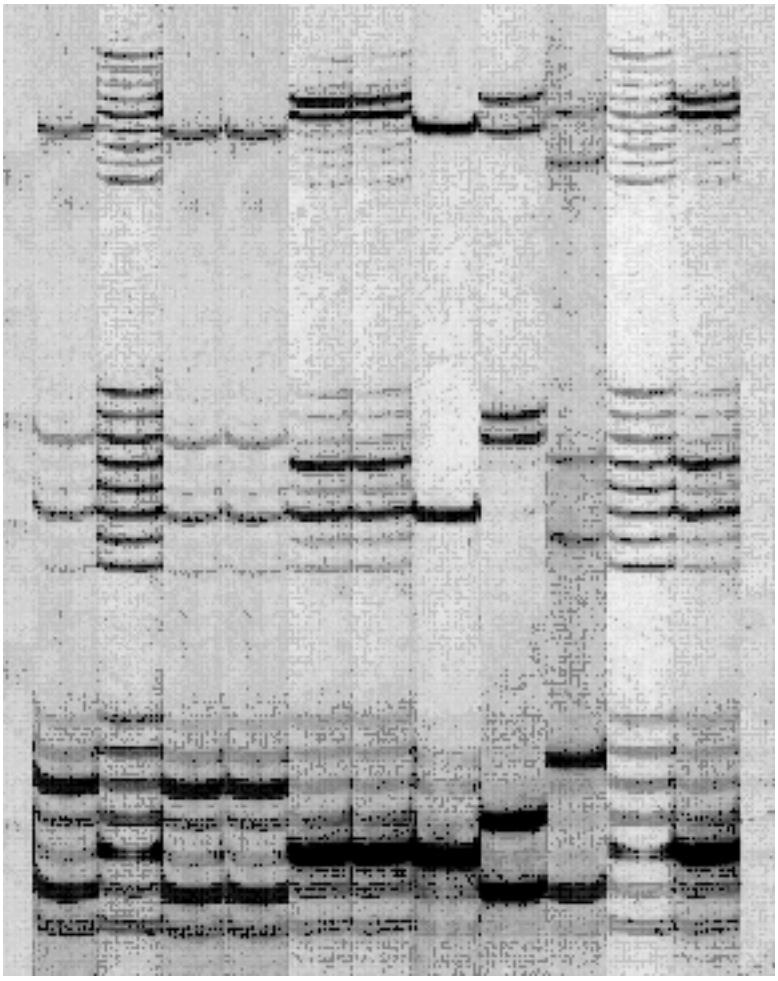

Fig (2): Case no 2

Interpretation of results

- DNA profiles of samples in lanes; 1, 3, and 4 have the typing: 6-9, 8-11 and 10-10 for the genetic loci: THO1, TPOX, & CSF1PO respectively. This indicates that the blood samples taken from the knife wound, the blood pool and from one cigarette butt all belong to the victim.
- DNA profiles of samples in lanes; 5, 6, & 11 have the typing: 7-7, 8-10, 11-12 for the genetic loci: THO1, TPOX, & CSF1PO respectively. Therefore; the second cigarette butt found on the table and the hair lock found in the right hand fingers of the victim's corpse belong to suspect number 4.
- DNA profiles in lanes no 7, 8 and 9 have the typing: 7-7, 8-8, 10-10, 6-8, 11-12, 10-12 and 6-9.3, 7-10, 8-11 which belong to suspects 1, 2 and 3 respectively. None of these DNA fingerprints were detected in the crime scene.

Investigation authorities concluded that suspects 1,2 and 3 were innocent and immediately liberated them. Police investigators faced suspect 4 with the DNA typing results and advised him to confess to improve his legal situation in the case or otherwise greater number of genetic loci will be utilized to provide perfect evidence to the court. At this point the suspect confessed of committing the crime which has been taken in consideration later on by the judge.

CASE NO 3

Crime scenario

A corpse of a strangled naked woman was found laid on the bed in her apartment where she lives alone. There were traces of bloody injuries in the chest, on the face and in the neck showing that she was strangled. Also signs of sexual assault that happened shortly before the death were noticed. Blood samples from a deep wound in the victim's neck and a vaginal swab were taken for examination. Criminal inquiry pointed to two delivery men of either the grocery or the pharmacy.

DNA Analysis

A sample of blood was taken from each of those two accused persons and transformed to the criminal lab to be examined and analyzed in comparison to the samples taken from the crime scene.
DNA profiling test was carried out on the two samples taken from the corpse of the victim and those taken from the two accused persons simultaneously using the CTT multiplex. Results are shown in figure (3).

The lanes contained the following samples:

- Lane 1: CTT allelic ladder
- Lane 2: blood sample taken from the grocery delivery servant.
- Lane 3: blood sample taken from the pharmacy delivery servant.
- Lane 4: blood sample taken from a wound in the neck of the corpse.
- Lane 5: vaginal swab taken from the corpse.
- Lane 6: CTT allelic ladder**.**

Interpretation of results

- DNA profile of the sample lane 5 is 7-8-9, 8-8, 10-11-12 for genetic loci: THO1, TPOX, & CSF1PO respectively, which represents that it contains more than one human nuclear genome to more than one individual.

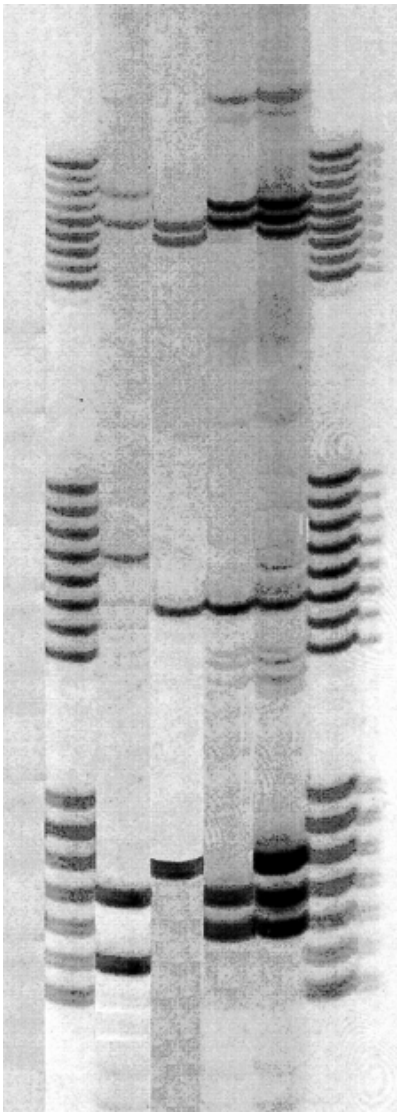

Fig (3): Case no 3

- DNA profile of the sample in lane 4 is 7-8, 8-8, 11-12 for genetic loci: THO1, TPOX, & CSF1PO respectively, which identifies the victim's profile.
- DNA profile of the sample in lane 3 is 9-9, 8-8, 10-11 for genetic loci: THO1, TPOX, & CSF1PO respectively. which identifies the CTT of suspect 1(pharmacy delivery man)
- DNA profile of the sample in lane 2 is 6-8, 10-10, 11-13 for genetic loci: THO1, TPOX, & CSF1PO respectively. which identifies the CTT of suspect 2 (grocery delivery man).
- Therefore; by excluding the typing result of the sample in lane 4 taken from a wound in the victim from that in lane 5 taken as a vaginal swab from the corpse, it was conclude that typing of the criminal's DNA profile found in his seminal fluid excluded suspect 2 who was immediately freed, while suspect 1 was arrested.

The accused felon did not take much time to confess that he raped the woman and killed her being convinced that he could improve his legal situation in the case and reduce the verdict emitted by the court.

Case no 4

Crime scenario

A criminal decided to kill his wife. He slaughtered her neck , cut her corpse into parts and put each part inside a plastic bag then discarded the bags in the desert. After he carefully mopped all traces left at crime scene. Few days later the victim's father reported his daughter's absence to police and accusing her husband. When investigating the conjugal home the criminal lab men found no signs of violence except for a tiny piece of bone that was found hanged at the cesspool.

DNA Analysis

Blood samples were taken from the victim's parents and CTT profiling was carried out simultaneously on parents` samples together with the bone sample found in the bathroom. Results of CTT print are shown in figure (4).

The lanes contain the following samples:

- Lane 1: CTT allelic ladder.
- Lane 2: blood sample taken from the mother of the victim.
- Lane 3: bone sample found at the crime scene.
- Lane 4: blood sample taken from the father of the victim.
- Lane 5: CTT allelic ladder.

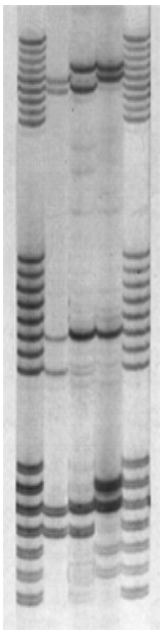

Fig (4): Case no 4

Interpretation of results

- CTT print in lane 2 is 8-9, 6-8, 10-11 for loci: THO1, TPOX, & CSF1PO respectively.
- CTT print in lane 3 is 8-9, 8-8, 10-12 for loci: THO1, TPOX, & CSF1PO respectively.
- CTT print in lane 4 is 9-10, 8-8, 11-12 for loci: THO1, TPOX, & CSF1PO respectively.
- This indicates that the tissue sample found at crime scene shares a common allele with the father and the mother of victim thus suggesting that the such tiny piece of tissue may belong to their missing daughter.

Police investigators confronted the husband with the DNA typing results asking him to explain how his wife's bone was hanged at the cesspool of bathroom.. They advised him to confess or other wise scientific evidences will be completed and presented to court besides the accusation report provided by the victim's father. The accused husband reported a complete confession during the investigation and narrated how and why he committed the crime.

Case 5

Scenario

A married man accused his wife of mistakenly ascribing her new born to him. He proved that he was abroad when pregnancy occurred and accused his wife's cousin for being the true father of the child. When investigators
arraigned the wife and her cousin for in questing both denied the impeachment of the husband. Buccal swap samples were taken from the child as well as from the husband, his wife and her cousin and simultaneously analyzed for CTT print.

DNA Analysis

Results of CTT profiling test are shown in figure (5).

The lanes contain the following samples:

- Lane 1: CTT allelic ladder
- Lane 2: CTT print of the wife.
- Lane 3: CTT print of the disputed child.
- Lane 4: CTT print of the alleged father.
- Lane 5: CTT print of the accused father.
- Lane 6: CTT allelic ladder**.**

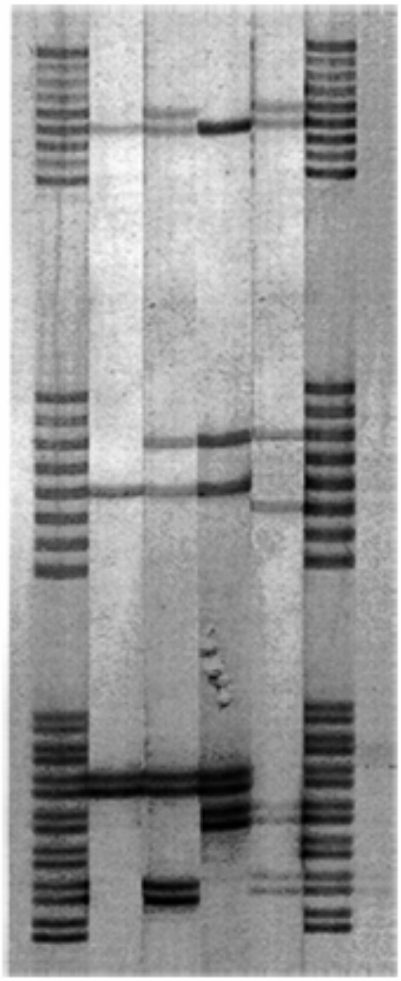

Fig (5): Paternity case

Interpretation of results

- CTT profile in lane 2 is 9-9, 9-9, 10-10 for the genetic loci: THO1, TPOX, & CSF1PO respectively.
- CTT profile in lane 3 is 6-9, 9-11, 10-11 for the genetic loci: THO1, TPOX, & CSF1PO respectively.
- This indicates that the child shares one allele in each genetic locus with that of the wife thus concluding that the biological father must contain the allele (**6**) in the **THO1** locus, the allele (**11**) in the **TPOX** locus and the allele (**11**) in the **CSF1PO** locus.
- However, CTT profile in lane 4 is 8-9, 9-11, 10-10 for the genetic loci: THO1, TPOX, & CSF1PO respectively.
- Therefore; the alleged father (husband) cannot be the biological father of the child, **thus establishing an obvious adultery case.**
- On the other hand, CTT profile in lane 5 is 6-8, 8-11, 10-11 for the genetic loci: THO1, TPOX, & CSF1PO respectively.
- **Therefore; the wife's cousin may be the biological father of the child an assumption that had been confirmed later on by comparing 16 different STR loci.**

Investigators confronted the wife and her cousin with the typing results which proved that the husband can not be the biological father of the child and advised the cousin to assign the child to his name to avoid being sentenced by the court. Upon writing his official confession the case was closed without having a trial.

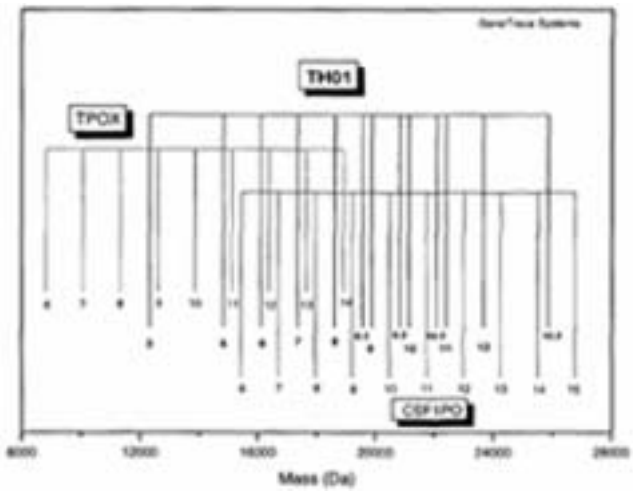

Fig (6): Schematic of expected allele masses for a CSF1PO-TPOX-TH01 (CTT) multiplex involving overlapping allele size ranges. All known alleles are fully distinguishable by mass with this interleaving approach (Butler *et al*., 1997).

DISCUSSION

There are literally hundreds of STR systems which have been mapped throughout the human genome. Several dozens of systems have been investigated for application in forensic purposes. The CTT print is one of the most polymorphic STR multiplex systems included in the 13 core STR loci designated by the Federal Bureau of Investigations (FBI) in the USA. The expected masses for the CTT multiplex including the STR loci CSF1PO, TPOX, and THO1 are schematically displayed in fig (6) (Hammond *et al.* , 1994). .All known alleles for those loci are fully resolvable and are far enough apart to be accurately determined. For example THO1 alleles 9.3 and 10 fall between CSF1PO alleles 10 and 11. For all three STRs in the CTT multiplex, the AATG repeat represents the building unit, which means that alleles within the same STR system differ by at least 1260 Daltons (Denise,1996). This helps in presenting a clear DNA typing result to the court, without explaining the scientific molecular techniques used in evaluating the result. An important reason for utilizing CTT print is the relatively great genetic diversity of the three loci in Egyptians as CSF1PO locus has 8 alleles with frequency range of 0.003 – 0.335, while TPOX locus contains 7 alleles with frequency varying from 0.004 – 0.492 and THO1 locus has 6 alleles with a frequency range of 0.028-0.330 (Refaat,2005). In those forensic application where more than a single person are suspected of committing the crime the investigators need to obtain a rapid and in expensive evidence to exclude innocent suspects and push true offenders to confession, the master of evidences. In this article, the significance of using the CTT multiplex in various applications is clarified through the presentation of five different legal cases (forensics and paternity) in which different biological sources of DNA were involved.

The first case was very mysterious and difficult to solve since the offender seemed to be experienced in this type of crimes. Therefore police investigators decided to focus their CTT screening on felons with criminal records of committing similar robberies and on police staff members for whom CTT data bases were already available. To do rapid inexpensive screening on huge number of suspects CTT print was sufficient to exclude subjects with no relation with the blood stain left at the crime scene. Such typing indicated that none of the felons with criminal records of robbing safes had the same CTT. Then it was decided to launch massive screening of a large number of inhabitants in the village using CTT print to reduce costs

and time when compared with the CODIS system. The results showed no similarities with the CTT of blood on the safe. Finally, when the later CTT was compared with the police staff data base the investigators were able to pinpoint the true offender who gained huge experience in criminal inquiry secrets through his work as a policeman. Needless to say CODIS analysis was required to present a successful case to court when the offender denied the charge.

In the second case it was necessary to free those suspects who have no materialistic linkage to the crime. The availability of diverse sources of DNA at crime scene has facilitated the process of cornering the offender with multiple evidences simultaneously since CTT multiplex results showed identical prints between the offender's DNA and those left in the victim's hands as hair lock and on the table as cigarette butts

In the third case analysis of the victim's vaginal swab was the key to solve the case through which the CTT print of the offender was concluded by subtracting the victim's CTT print (taken from the corpse) from the mixed CTT prints in the vaginal swab and, therefore, having no evidence to keep the other suspect under custody. Therefore, CTT Multiplex is thought to provide a powerful and unique materialistic evidence through the analysis of a post coital mixed stain providing a decision that the grocery man is not guilty and represents a scientific pressure tool which obligated the true offender to confess or otherwise further analysis would be carried out on the extracted DNA samples using larger number of STR loci reaching the assertion state that proves that he is the true offender.

In the fourth case the missing corpse (the major materialistic evidence of a murder crime) could complicate the case because the offender was very careful to hide all traces of violence and the subsequent failure of police investigators to prove the crime of murder. The very tiny (even microscopic) piece of human hard tissue that was recovered from the crime scene could belong to the husband himself or even could be a non human tissue. The later assumption was denied via the biological investigations which had been carried out on this sample and indicated that it is from a human origin. The CTT profiling test could easily exclude that it belongs to the husband's genome. Again a rapid comparative CTT profiling of both the victim's parents and the piece of bone directed the police investigators` attention towards a strong possibility that a case of murder had happened in the conjugal apartment and the husband's confession was a natural result of evidential confrontation from criminal inquiry men.

In the paternity case, CTT print of the disputed child and the alleged father (husband) was sufficient to exclude paternal relation between them thus establishing an obvious adultery case for which the wife should be sentenced according to the Egyptian law. On the other hand the concordance of CTT print of the wife's cousin (accused father) with the child's CTT print opened the gate for friendly dialog between police investigators and him to confess and assign the child for his name or otherwise will be subjected to CODIS analysis and face the verdict emitted by the court.

CONCLUSION

We conclude that CSF1PO, TPOX, TH01 genetic loci are genetically heterogeneous so that they are useful in certain forensic applications. The (CTT) multiplex can act as a scientific pressure on accused persons to confess and simultaneously can exclude other innocent persons who may face injustice being arrested for long time during criminal investigations.

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