



Dried Umbilical Cord and Cord Blood Sample: Reliable Non Invasive Method of Sample Collection than Usual Invasive Method from Neonates for Forensic Identification

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Case Report

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Abstract

Forensic DNA profiling has an important role on the society by providing reliable evidence whether a man is the biological father of an individual or not. To do that biological samples are required for DNA analysis. It is very easy to collect blood samples from the adults but at the same time quite difficult to draw blood from neonates. It requires lot of skill as the process is invasive and many of the time collected amount is very few. To forbid that issue we have successfully isolated genomic DNA from a new born in a non-invasive way, by collecting the dried umbilical cord and the cord blood sample. Matching or exclusions between alleged parents and the child were established with the help of STR typing.

Keywords: Forensic; Disputed paternity; DNA analysis; Non-invasive; Umbilical cord; STR typing

Introduction

Forensic DNA fingerprinting can be defined as the comparison of the DNA in a person's nucleated cells with that identified in biological matter found at the scene of a crime or with the DNA of another person for the purpose of identification or exclusion [1]. It is a useful tool for verifying a stated biological relationship like paternity or maternity [2]. It is the most accurate and widely acceptable technology to establish the biological relationship among two or more individual. Till date the preferred forensic specimen collection technique for DNA testing is blood sample either in liquid form or stain forms [3]. For collection of intra venous blood samples from the accused or the victim either direct liquid blood is obtained or bloodstains are made using lancet on FTA cards [4]. But cases related with exhibit sample collection from newborn babies, the methods are always difficult and painful. Venipuncture is the method of choice for blood sampling in term of neonates; however, it

is complex and requires an experienced, knowledgeable and trained phlebotomist [5]. On the other hand umbilical cord blood is the blood left over in the placenta and in the umbilical cord after the birth of the baby. This cord blood is composed of all the elements found in whole blood. It contains red blood cells, white blood cells, plasma, platelets and some other hematopoietic stem cells [6-8]. During prenatal development, the umbilical cord is physiologically and genetically part of the foetus and, (in humans), normally contains two arteries (the umbilical arteries) and one vein (the umbilical vein), buried within Wharton's jelly [9-11]. The umbilical vein supplies the foetus with oxygenated, nutrient-rich blood from the placenta. Conversely, the foetal heart pumps deoxygenated, nutrient-depleted blood through the umbilical arteries back to the placenta [12,13].

The objective of this study was to solve the disputed paternity cases by using the DNA yield from dried umbilical cord or cord blood samples and other blood/bone samples to

regularize the noninvasive sampling methods for neonates in forensic routine sample collection techniques.

Case Study 1: A person was arrested and recommended jail custody for the allegation of a rape. A baby was delivered by the lady. Unfortunately the baby was expired within a few days and the body was buried under the soil. Police can only recover one small bone piece after few days. One small dry umbilical cord tissue of the baby was also recovered from the lady's house. DNA profiling is done to identify the real biological father of the deceased baby.

Case Study 2: A person was arrested by police for allegation of a rape. The cord blood sample of the stillborn baby of the victim was collected and preserved by the doctor. DNA profiling is done to identify the actual biological father of the stillborn baby.

Materials and Methods

Exhibit samples of Case Study 1: The image of each case exhibits from case study 1 are given below in Figures 1-4.

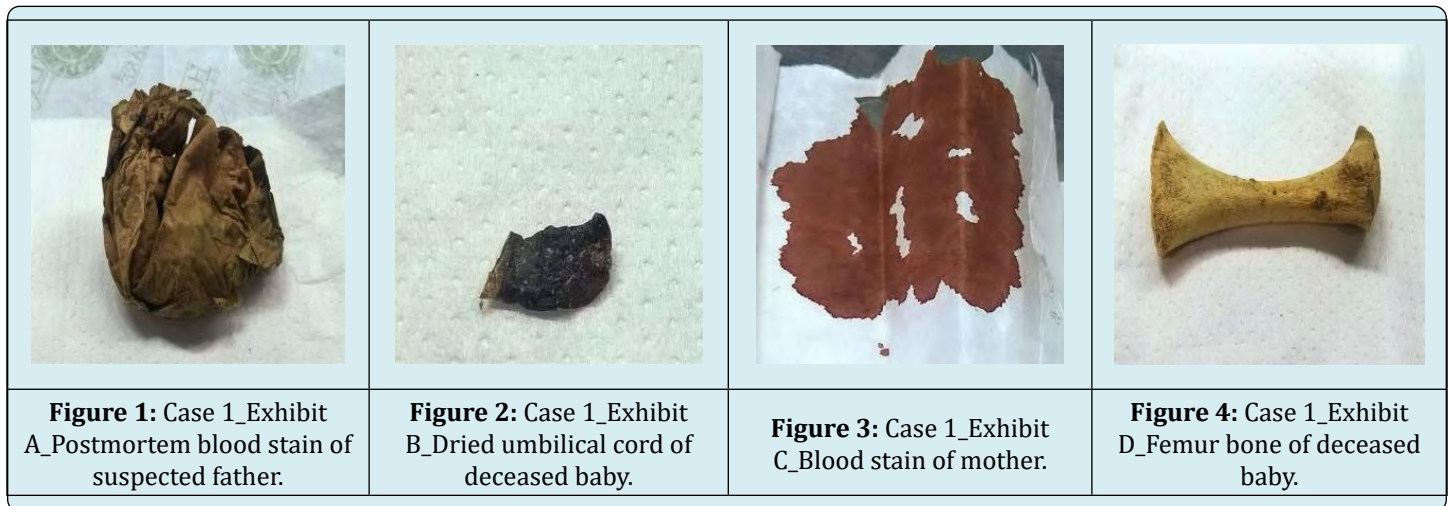
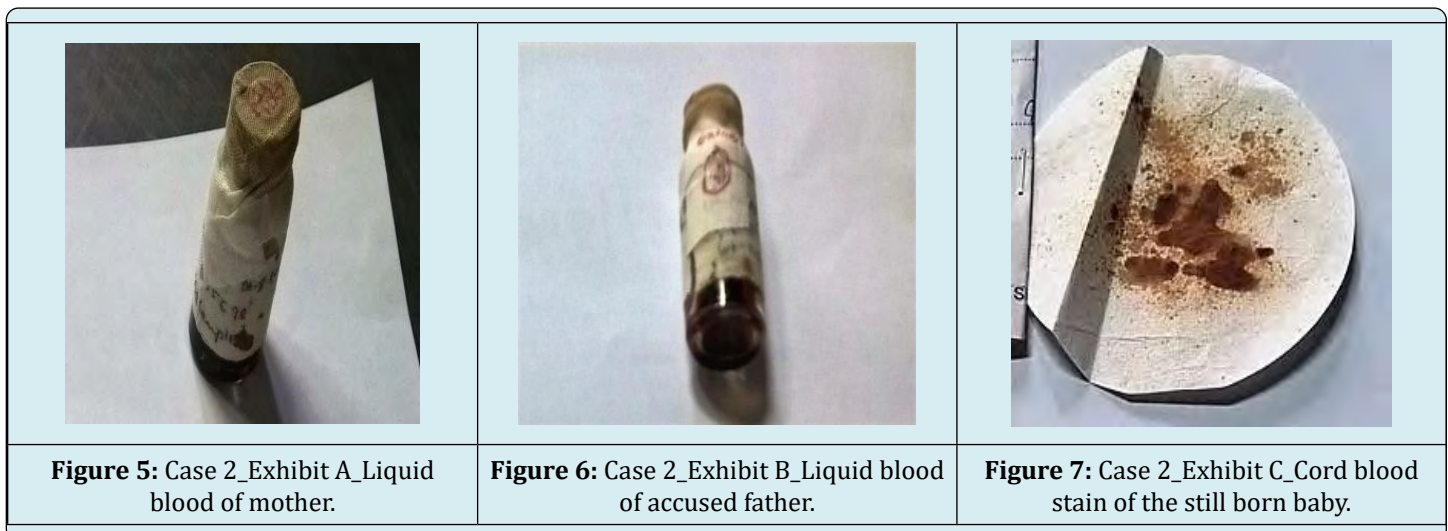


Exhibit samples of Case Study 2: The image of each case exhibits from case study 2 are given below in Figures 5-7.



Procedure for Isolation of Total DNA from Dried Umbilical Cord

Genomic DNA was isolated from dried umbilical cord tissue using Stain Extraction Buffer (SEB) followed by Amicon purification. The amount of dried umbilical cord was merely 1 inch in length. To isolate genomic DNA from it, at first cut a small part from the dried cord and then wash the cord with DNase free water to rehydrate it. When it becomes soften to cut in more small pieces take the rehydrated cord and cut them small, try to choose darker part of the cord and put in a 1.5ml eppendorf tube [14,15]. Now, with the help of a pipette just smash the pieces lightly and add 400 μ l stain

extraction buffer into it followed by 20 μ l of Proteinase K. Incubate at 56° over night.

From liquid blood and cord blood stain DNA was extracted using Phenol-Chloroform method followed by ethanol precipitation [16] and from femur bone using organic method and Amicon purification.

Analysis of Isolated DNA

Quality and quantity of isolated DNA was checked through 1% TAE gel electrophoresis.



Figure 8:- The 1% Agarose gel electrophoresis results of isolated DNA Case-1 (Ex-A- Post mortem blood stain of suspected father, Ex-B- Dried umbilical cord of deceased baby, Ex-C- Blood stain of mother, Ex-D- Femur bone of deceased baby); “+ve” Std=20ng/l; “-ve” Std; Case-2 (Ex-A- Liquid blood of mother, Ex-B- Liquid blood of suspected father, Ex-C- Cord blood stain of the still born baby).

PCR Amplification and Sequencing Analysis

For Case 1 the isolated DNA PCR amplifications was performed using Investigator® IDplex Plus kit [17] and for Case 2 it was performed using AmpFISTR®Identifiler®PCR Amplification Kit [18] which co-amplifies 15 autosomal STRs

and a sex determination marker amelogenin in GeneAmp® PCR System 9700 (Applied Biosystems, Fosterb City, CA, USA) [19] with the following cycling parameters given in table 1. The PCR products were then sequenced in ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Fosterb City, CA, USA) using GeneMapper ver 3.5 software [20,21].

PCR Cycle	Investigator®IDplex Plus Kit	AmpFISTR®Identifiler®PCR Amplification Kit
Initial incubation step	95°C for 5 min	95°C for 11 minute
Denature	96°C for 10sec (30 cycles)	94°C for 20sec (29 cycles)
Anneal	61°C for 2 min.(30 cycles)	59°C for 3 min (29 cycles)
Extension	72°C for 0 min.	72°C for 0 min
Final extension	NA	60°C for 10 min
Final hold	10°C for ∞	4°C for ∞

Table 1: PCR Cycle Conditions.

Results and Discussions

The resultant allele distributions for the studied loci with both the case exhibits are shown in the table below and the electropherograms are given at the end.

Discussion of Case 1

The genetic profile of Exhibit: B (Dried umbilical cord of deceased baby) and Exhibit -D (Bone of Deceased Baby) are identical at all the amplified loci. The genetic profile of the Exhibit C (Blood stain) is consistent as the biological mother

of the Exhibit B and D at all the amplified loci but the Exhibit A (Blood stain) is not the biological father of the Exhibit B and D.

Markers	CASE 1				CASE 2		
	Post mortem blood stain of suspected father	Dried umbilical cord of deceased baby	Blood stain of mother	Bone of Deceased Baby	Liquid Blood of Mother	Liquid Blood of Suspected Father	Cord blood stain of the still born baby
Amelogenin	X, Y	X,X	X, X	X, X	X, X	X, Y	X, X
TH01	6, 9	8, 9	8, 9	8, 9	6,7	6	6,7
D3S1358	15, 16	16, 17	17	16, 17	18, 19	14, 17	17, 18
vWA	17	14	14	14	14,16	18	14, 18
D21S11	32,2	29, 31	28, 29	29, 31	29	28, 29	29
TPOX	8, 11	8, 9	9, 11	8, 9	11	8, 12	11, 12
D7S820	11, 12	11, 12	9, 11	11, 12	7, 12	10, 11	7, 10
D19S433	14, 15.2	13.2, 15.2	12.2, 13.2	13.2, 15.2	13, 15.2	13, 15	13, 15
D5S818	11, 12	11, 12	11, 13	11	11, 12	11	11
D2S1338	21	19, 21	19	19, 21	18, 21	18, 19	18
D16S539	10, 12	10, 14	10, 12	10, 14	11, 13	12	12, 13
CSF1PO	11	12	10, 12	12	11, 12	11,12	11
D13S317	8	11, 14	11	11, 14	11, 13	11,12	11
FGA	24, 25	23, 24	22, 23	23, 24	21, 22	22, 24	21, 22
D18S51	14, 20	15	14, 15	15	13, 15	14,15	15
D8S1179	10	11, 15	8, 11	NA	11, 15	12, 13	11,13

Table 2: Allele distributions for the studied loci of both the cases.

Discussion of Case 2

The genetic profile of Exhibit: C (Cord blood stain of the

Baby) is consistent as the biological daughter of the Exhibit: A (Liquid blood of the mother) and Exhibit: B (Liquid blood of the suspected father) at all of the amplified loci.

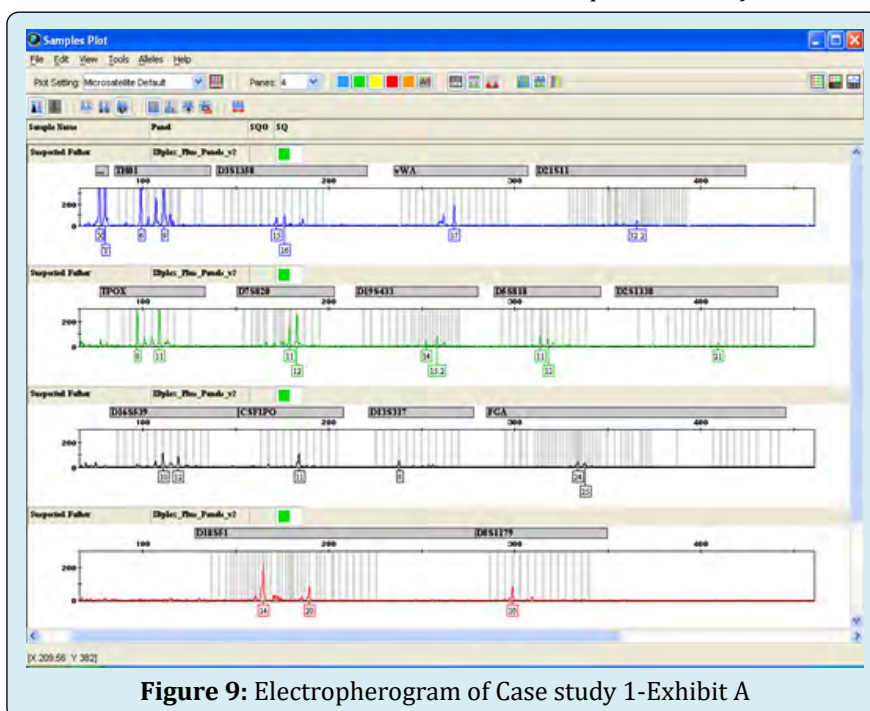


Figure 9: Electropherogram of Case study 1-Exhibit A

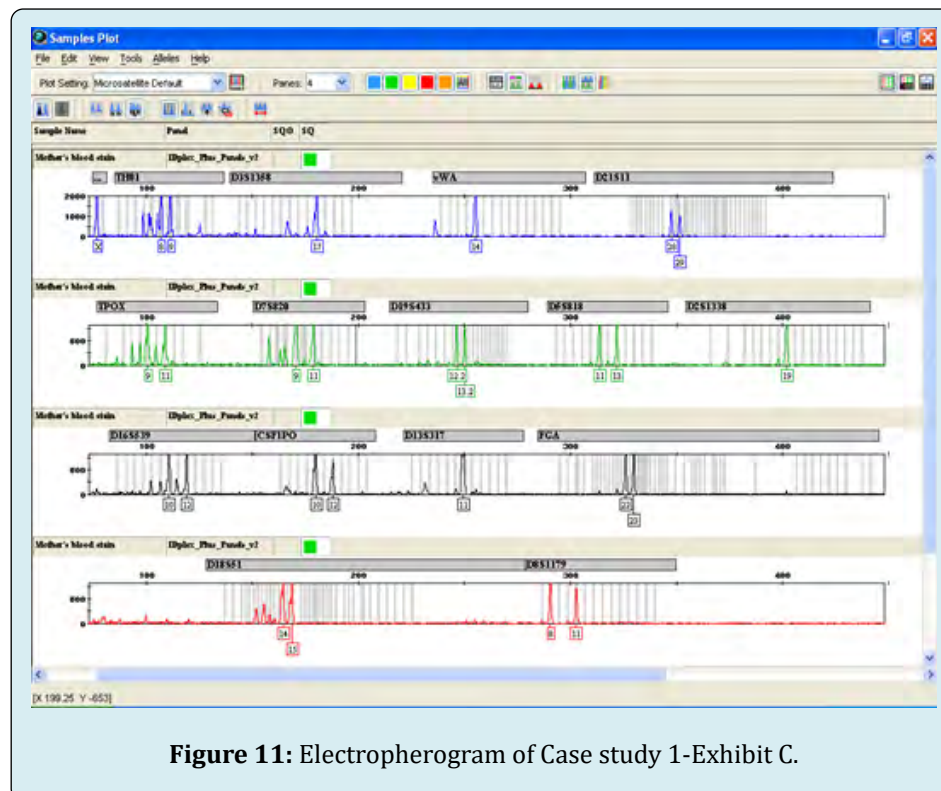
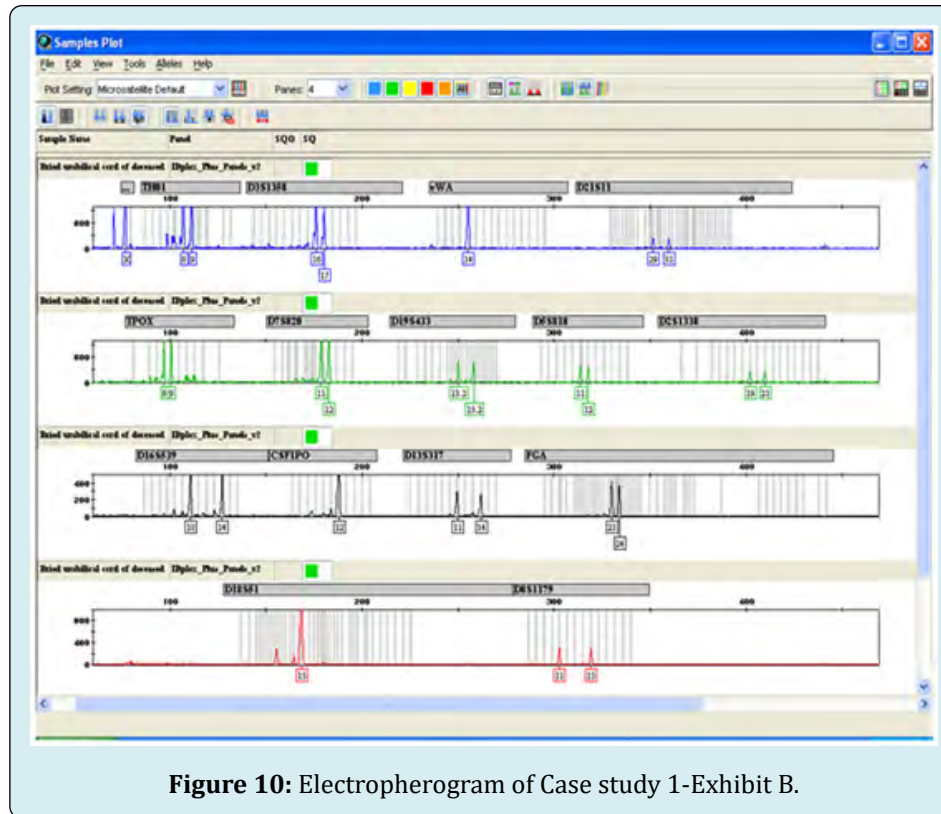




Figure 12: Electropherogram of Case study 1-Exhibit D.



Figure 13: Electropherogram of Case study 2-Exhibit A.



Figure 14: Electropherogram of Case study 2-Exhibit B.



Figure 15: Electropherogram of Case study 2-Exhibit C.

Conclusion

In this study we found statistically significant similarities in both genetic profiles between the dried umbilical cord tissue, cord blood sample and bone sample. We also observed that the bone sample did not yield a suitable amount of DNA for a generation of complete profile. This study established that both dried umbilical cord and cord blood of the still born baby can be used as alternatives to whole blood or dry blood spots for genomic DNA extraction from premature infants. Dried umbilical cord or cord blood samples are also a suitable source for newborn biological relationship establishment. Where blood sample collection is not possible, dried umbilical cord or cord blood samples may be an acceptable and alternative non invasive procedure to obtain neonatal DNA. This sampling method must be regularized during routine forensic sample collection of neonates.

Conflicts of Interest

The authors declare no conflicts of interest.

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- GeneAmp® PCR System 9700 - Applied Biosystems. User's Manual.
- ABI Prism 3100 Genetic Analyzer. Applied Biosystems. User's Manual.
- GeneMapperID v 3.2 software.

