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Original Article

Recovery of High Molecular Weight DNA from Old viscera Samples via Two Established Methods for Human Identification: A Comparative Study

B.K. Mohapatra[1], U.S. Thakur[2], K. Chauhan[3], A. Sharma[4], S. Dagar[5], R. Kaushik[6] and C. Behera[7]*

[1]Principal Scientific Officer, [2]Senior Scientific, [3]Senior Scientific Officer II, [4,5]Laboratory Technician, Assistant Biology division, Central Forensic Science Laboratory (CBI), New Delhi, India
[6]PhD Student, [7]Associate Professor, Department of Forensic Medicine, AIIMS, New Delhi, India
*Corresponding author email id: drchitta75@ rediffmail.com

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ABSTRACT

At autopsy, in cases of suspected poisoning or drug intoxication, the viscera are mostly preserved in saturated common salt solution for toxicological analysis. However, frequent situation arises where the preserved viscera sample gets questioned for its source of origin and identification of the individual contributing the said viscera becomes important. In such a scenario, to identify the source of viscera, the DNA Profiling technique can be employed. This study aims at recovery of high molecular weight DNA suitable for DNA profiling from a 5-year-old viscera sample through modified validated methods. Approximately 2 g of viscera samples (kidney) were taken for the study and two different established DNA extraction methods were employed for isolating DNA for downstream procedures viz. quantitation of the extracted DNA, the amplification of the desired loci Polymerase chain reaction (PCR) and its subsequent genotyping. The yield of DNA extracted from both the methods was quantitated using real time PCR system and a complete DNA profile was obtained for the purpose of comparison with the biological relatives of the individual.

Keywords: Viscera, PCR amplification, DNA profiling, Organic extraction, Extraction using EZ1 bio-robot

INTRODUCTION

The identity of the source of viscera, that is, it is of the deceased or not; becomes important when the preserved viscera of the individual become questioned. Even though the individualisation is possible via DNA profiling technique, the real challenge involved is selection of particular tissue material from very old viscera samples and extraction of high molecular weight DNA from the said viscera samples.

Normally the viscera samples in Indian mortuaries are preserved in saturated common salt (NaCl) solutions and handed over to the investigating agencies for onward transmission to the forensic laboratories for chemical and toxicological analysis. Under normal circumstances the viscera samples are kept in the malkhana of police stations for many days in normal condition at room temperatures and then submitted to the FSL/CFSL for analysis. After a chemical or toxicological examination of the viscera, it is again handed over to the investigating agency, where it is kept under normal climatic conditions at room temperature for many years. Blood, long bones, teeth are the common source to establish the identity of individuals using DNA-profiling technique.[1–4]

Establishment of identity from viscera sample via DNA profiling being a secondary attempt and is always done only when the identity of the sample becomes questioned in the court of law. After a long time since the collection/ preservation of the viscera of the deceased in the mortuaries, forensic analysis of human DNA in such old viscera samples can be a challenging task. Factors like degradation and/or putrefaction results in low molecular weight DNA that can affect the analysis due to the lack of quality material required in the downstream assays.[5,6] Extracting a good quality of DNA from old preserved and putrefied samples is always a difficult task, it requires a careful examination of samples and deciding which method would be appropriate for extraction.

This study is an attempt to recover good quality DNA in desired quantities from 5-year-old viscera samples, by two separate established DNA extraction methods with suitable modifications and its subsequent DNA Profiling. The viscera sample was referred to our laboratory (Central Forensic Science laboratory, Central Bureau of Investigation) for the purpose of DNA Profiling and comparison with living biological relatives of the deceased. The postmortem of the deceased in the current case was conducted during the year 2011 and the samples were kept in the Police station malkhana under normal climatic conditions at room temperature.

MATERIAL AND METHODS

From the available viscera (Figure 1), 2 g of visceral tissue (kidney), preferably selected via the presence of fibrous muscles, were cut and taken in two separate 15 ml falcon tubes. The tissues were further cut into small pieces and washed with distilled water (Milli Q water) repeatedly with gentle vortexing. Further the tissue samples were kept dipped in the distilled water (Milli Q water) for 1 day (24 hours) so as to allow all the available salts to dissolve out. This process of washing was continued for two more days for complete removal of the available salts as the salts can act as a strong inhibitor during PCR amplification.

Two separate DNA isolation methods were employed for the extraction of DNA from these samples.

i. Organic DNA extraction method, that is, Phenol-Chloroform extraction method[7];

ii. Extraction using EZ1 Bio-Robot by utilising prefilled Qiagen DNA extraction cartridges for tissue samples.

The DNA extracted via organic method was further subjected to purification using column-based purification kits supplied by the Zymo Research Corp, USA.

The quantification of extracted DNA was done using Real Time PCR system (Model 7500: Applied Biosystems). The extracted DNA was further subjected to multiplex PCR amplification for 15 STR loci and amelogenin using the AmpFlSTR Identifiler Plus Kit using PCR Model 9700 (ABI) (Figure 2). Subsequent genotyping of the amplified products were carried out using ABI Prism 3130XL genetic analyzer. A complete genotype plot was obtained from the DNA extracted via both the described methods (Figures 3 and 4).

RESULTS

High molecular weight amplifiable DNA was obtained from both the extraction methods employed for DNA extraction with an increased quantity being yielded EZ1 Bio-Robot automated DNA extraction system utilising prefilled DNA extraction cartridges (Table 1).

On the basis of comparison of the quality of peaks in the genotype plots the amplified DNA obtained from the DNA extracted via automated DNA extraction system was found to be more encouraging in comparison to the DNA extracted via organic method.

DISCUSSION

DNA profiling is an established technique used world wide for the confirmation of identity, in any kind of identity disputes. The extraction of DNA is possible from

Table 1: Quantitation of Extracted DNA Samples using RT-PCR

Sample	Quantity (ng/µl)
Ex-1_EZ1	1.11
Ex-1_Organic	0.48

Figure 1: Photograph of Viscera Sample

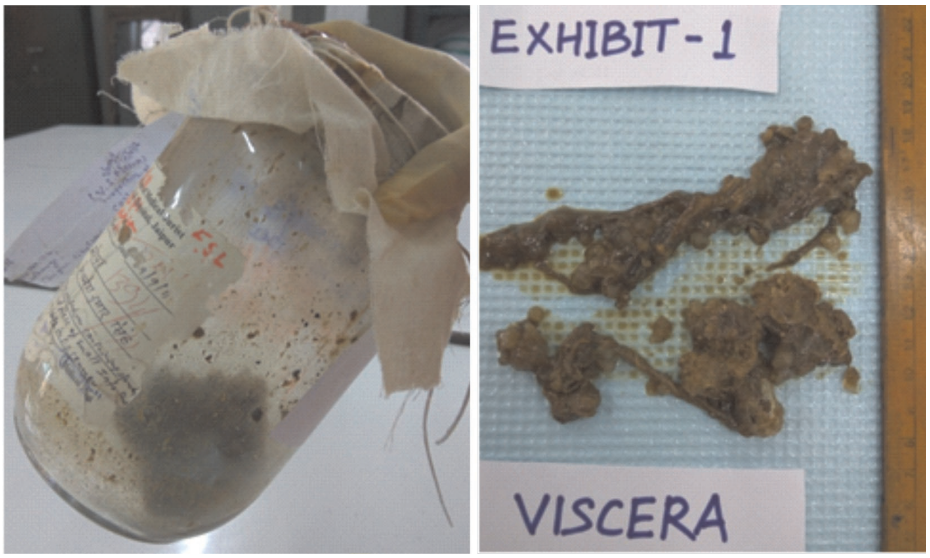
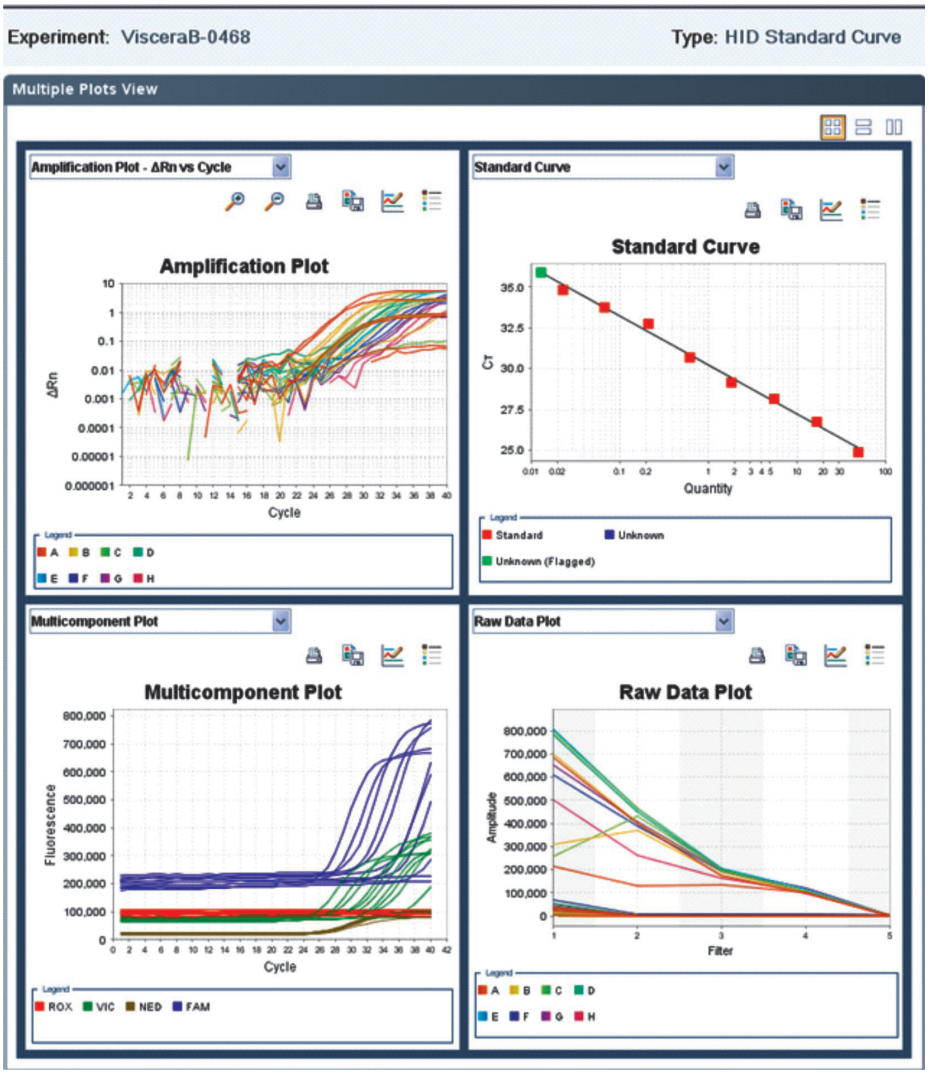


Figure 2: Multiple Plots Generated from RT-PCR



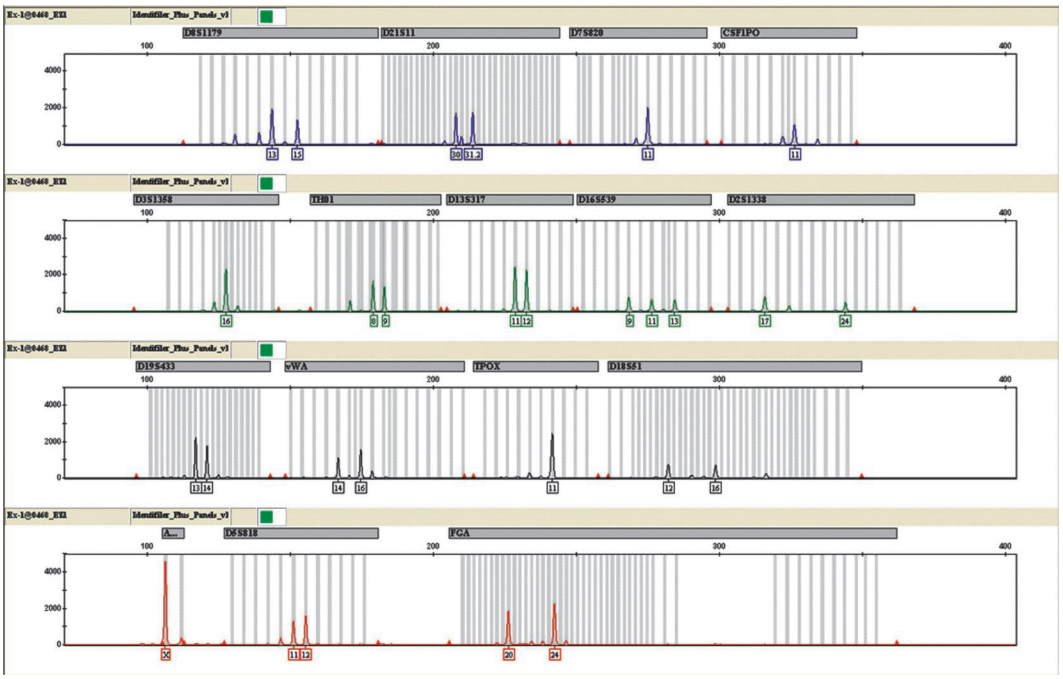


Figure 3: Electropherogram of DNA Extracted from Ex-1 using EZ1 DNA Extraction Method

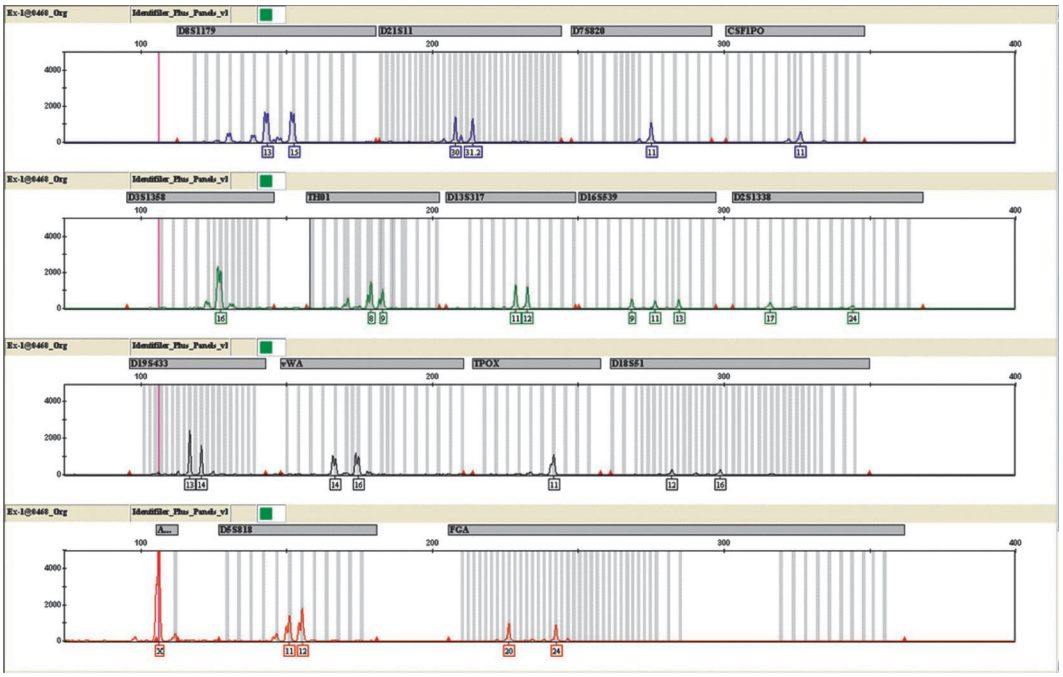


Figure 4: Electropherogram of DNA Extracted from Ex-1 using Organic Extraction Method

nucleated cells of the body; however, the condition and quantity of these bodily tissues determine the quality and quantity of DNA obtained after extraction.

Determination of the quantity and quality of human DNA extracted from biological evidence is vital for numerous reasons. Firstly, it depends on the source and extraction method used. This affects the downstream method as the quantity of input DNA and its relative length can determine which genotyping procedure to be used like short-tandem repeat (STR) typing, mini-STR typing or mitochondrial DNA sequencing. Secondly, in forensic analysis, it is important to preserve as much of the

evidence as possible for retesting. Prior to utilising any destructive analytical method, it is also important to determine the total DNA amount available. Finally, results from preliminary quantitative and qualitative evaluations allow a more informed interpretation of downstream analytical results.[8]

Studies on quality and quantity estimation of DNA extracted from preserved putrefied samples have been reported earlier.[7,9] It is established that result yielding DNA can be recovered from many year old viscera samples using sensitive washing, purification and extraction methods. This could always be advantageous in human identification when the preserved samples of the deceased become questioned even after many years of chemical examination of viscera during long court proceedings as it is evident from this case study, where the identification of the source of viscera after 5 years of preservation was questioned and send to our laboratory to match the DNA profile with the relatives of the deceased.

The quantification after DNA extraction is an important step, which provides the information about the amount of DNA present in unknown samples. These data can be used successfully to obtain better quality results preserving the sample for further analysis.[10], [11] It was observed that a sufficient quantity of high molecular weight DNA was extracted in both the methods with a higher quantity of DNA, that is, being recovered via EZ1 Bio-Robot Automated DNA Extraction System utilising prefilled DNA extraction cartridges.

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