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Quantitative and qualitative study of STR DNA from ethanol and formalin fixed tissues

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ABSTRACT

Complete and concordant autosomal short tandem repeat (STR) DNA profiles were obtained from 2.0 mg human tissue samples of various types after they were preserved for 24 weeks in 100% ethanol and amplified with the GlobalFiler® and the PowerPlex® Fusion Amplification Kits. When 4.0 mg of the same tissues were preserved for 12 weeks in 10% Neutral Buffered Formalin (NBF) they yielded partial profiles when amplified with the same kits. However, these NBF preserved tissues yielded complete autosomal profiles when amplified with the *AmpFlSTR*® MiniFiler™ Amplification Kit.

Six tissue specimens from the male donor were also amplified with the PowerPlex® Y-23 System. Y-STR profiles were successfully generated from 2.0 mg tissue specimens when preserved for 12 weeks in 100% Ethanol. Only partial profiles were obtained when the fixation time was increased to 24 weeks. Only partial Y-STR profiles were also obtained from 4.0 mg tissue specimen from the same donor when preserved in 10% NBF.

In an attempt to optimize the method, the preserved samples that yielded partial profiles were homogenized using the BioMasher III disposable homogenizer and BioMasher III homogenizer and filter. These homogenized tissues did not yield significantly better or more complete profiles when using the GlobalFiler®, *AmpFlSTR*® MiniFiler™ Amplification Kits, the PowerPlex® Fusion System or the PowerPlex® Y23 System.

A total number of 240 tissue samples were analyzed in this project. The amplification of the tissues preserved in 10% NBF with kits such as *AmpFlSTR*® MiniFiler™ and GlobalFiler® Amplification Kits that contain mini STR primers can be beneficial in forensic testing. The results of the study indicate that in cases such as when a victim or a suspect is missing, the profiles obtained from minute amounts of chemically fixed tissues can be used as reference samples and compared to evidence found at the crime scene.

1. Introduction

Identification of individuals from body fluids found at crime scenes has been accomplished using molecular biology techniques such as STR analysis [1–5]. Additionally, such techniques are used in forensic pathology for identification and investigation of causes and manner of death [6]. However, postmortem specimen decomposition results in the breakdown of deoxyribonucleic Acid (DNA), ribonucleic Acid (RNA) and proteins, which may cause difficulties in using these specimens in routine forensic testing [7]. Additionally, mitochondrial and nuclear DNA in fixed tissues can undergo degradation due to the chemical reactions caused by fixatives [8]. The main objective of using fixatives is to prevent autolysis events and to preserve the physical feature of tissues; therefore, allowing disease diagnosis and cause of death investigations [9].

The physical and chemical mechanisms of fixatives fall into wide range of categories, including crosslinkers, coagulants, additives, dehydrants, and combinations of these categories [10,11]. This study focuses on two types of chemical fixatives: 10% NBF which is a cross-linker and 100% ethanol (a coagulant). NBF is one of the most commonly used fixatives in pathology laboratories because it fixes and preserves broad range of tissue types and tissue components [12]. It is widely used due to its high reactivity, ease of preparation, and lower cost [13]. However, studies have shown that formaldehyde causes DNA degradation due to the gradual formation of formic acid from formaldehyde, resulting in small fragments of DNA that are not suitable for

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molecular biology and forensic testing [14,15]. Exposure of specimens to formaldehyde can cause protein-DNA crosslinks, protein modifications and interstrand DNA crosslinks [16]. It has been reported that there are at least four types of interactions of DNA with formaldehyde, which is the active component in formalin [12]. First, the addition of formaldehyde to nucleic acid bases forms hydroxymethyl (methylol) groups (–CH_2 OH). This type of damage to nucleic acids is most common during fixation, and it can be reversed by applying a heating step in a buffered solution [17]. Second, nucleophilic attack of *N*-methylol, the primary form of formaldehyde in solution, on amino bases forming methylene bridge between the two adjacent amino groups. Third, apurinic and apyrimidinic sites can accumulate after formaldehyde exposure due to the hydrolysis of *N*-glycosylic bonds, which results in creating free pyrimidine and purine sites. Fourth, short chains of polydeoxyribose with pyrimidines attached to them can form via the slow hydrolysis of phosphodiester bonds induced by formaldehyde [12].

Ethanol or alcohol-based fixatives preserve tissues through coagulation rather than cross-linking [10]. When tissues are fixed in alcohol-based fixatives, the hydrophobic bonds are destabilized by the removal and replacement of free water in cells. Moreover, hydrophilic areas of the proteins are destabilized because of water removal. These chemical effects cause changes to the water solubility properties of proteins and their tertiary structure, which are mostly irreversible [10,18]. Previous studies reported that the alcohol-based fixatives preserve higher molecular weight DNA (>200 bp) and RNA, making these samples more suitable for Short Tandem Repeat (STR) typing in forensic applications than formalin-fixed tissues [19–21]. In contrast, buffered formalin preserves the histological structures of tissues better, making it more applicable for use in histological examinations [8].

Disrupting cells is an important step when extracting DNA and RNA. Research with the BioMasher disposable homogenizer showed that it is efficient in RNA extraction from various types of mouse and rat tissues. The BioMasher device is an easy to use device, available as sterilized tube, comes with a pestle and can eliminate cross-contamination between samples [22].

The objectives of this study were the following: the first was to compare the quantity and quality of DNA extracted from minute amounts of different types of tissues when preserved in 100% ethanol and 10% NBF over defined periods of time.

A second objective was to amplify minute amounts of these preserved tissue samples using various recently available STR Amplification Kits. Two of these autosomal Amplification Kits and one Y-STR kit were evaluated for their efficacy in generating STR profiles from minute amounts of tissues which have been preserved in chemicals for several weeks. The goal of this research was to evaluate the STR profiles and compare the results with another kit introduced in the forensic community several years ago for use with degraded samples. The results from all amplification were compared for concordance within and between various types of tissue samples obtained from the same donor.

The third objective included generating profiles from various types of tissues preserved in the two chemical fixatives to determine if particular specimens yield better quality STR profiles when compared to other types.

Finally, the fourth objective was to determine the efficacy of the mechanical homogenization using the BioMasher III disposable homogenizer in increasing profile recovery from tissues preserved in the two fixatives mentioned above.

2. Materials and methods

Blood and tissue samples were obtained from three-deceased individuals; two females and one male. A total of 16 tissue specimens from these three donors were used in this study. Five tissue types, namely small bowel, kidney, colon, liver and muscle were obtained from each individual. In addition, one gall bladder specimen was also obtained from the male donor. A total number of 240 tissue samples were used in this research, which includes 16 pristine tissue specimen as well as tissues fixed in the two fixatives indicated in Table 1. These tissues were kept frozen until needed for analysis. Three blood samples were collected in Vacutainer® tubes containing EDTA, a chelating agent that inhibits DNA degradation. As required by the policy of The Pennsylvania State University, all samples including the blood and tissues were obtained and processed following the guidelines approved by the Institutional Review Board (IRB) and Institutional Biosafety Committee (IBC). Every sample used in this study was anonymized.

2.1. Sample preparation

Each pristine blood and tissue sample was processed initially without adding any fixatives to it in order to establish concordance between and within samples obtained from each donor. DNA was extracted from 3 μL of blood from each donor and DNA profiles were generated (used as reference samples). 2.0 mg of each type of tissue was used for obtaining DNA profiles from the pristine tissue samples prior to adding any fixatives. Once these profiles were generated subsequent experiments with fixatives included 2.0 mg or 4.0 mg of each specimen.

Initially, experiments were performed to compare the yield of DNA from tissues incubated in both fixatives for 1 to 13 days. Liver tissue from a female deceased individual was cut and 2.0 mg of each sample was immersed in each of the two fixatives: 100% ethanol (Electron Microscopy Sciences, Hatfield, PA) and 10% NBF (Globe Scientific INC, Paramus, NJ, USA). The total number of tissues used at this stage was 20. Specimens were preserved in test tubes at room temperature. The test tubes were corked and sealed with Parafilm® to avoid evaporation.

Each of these liver tissues was preserved in each fixative in increments of 1 day. Thus, each liver sample (total of three) was extracted and quantified the day before each was immersed in one of the fixatives (day 0) and then every day during the duration of preservation. This process required three liver samples, preserved from day 0 to day 13 in two chemical fixatives. Thus, a total of 84 samples were extracted and quantified at this stage of the study.

After this stage of the study was completed and data was analyzed, each of the 16 tissue specimens from the three donors was cut, weighed and immersed in both of the two fixatives. Each 2.0 mg specimen was preserved in 100% ethanol for the length of time and incubation period described in Table 1. The duration of preservation in 100% ethanol ranged from 4 weeks to 24 weeks. Experiments using 10% NBF were performed with 4.0 mg of each tissue. Each of the 4.0 mg specimens was immersed in 10% NBF for a period of 4 weeks, 8 weeks, and 12 weeks. At the end of each of these periods, samples were taken out of each of the fixatives and DNA was extracted from them. A total of 16 tissue specimens from the three individuals were fixed in both chemical fixatives for each of the duration time mentioned in the table.

2.2. Tissue homogenization

The BioMasher III disposable homogenizer (DiagnoCine LLC, Hackensack, NJ) is composed of a filter tube, a collection tube and a pestle [23]. The filter tube contains a filter paper with 80–145 μm pore size. When tissue is added to the BioMasher III along with lysis buffer, it is homogenized through hard textures on the pestle and the filter tube with grinding motions by hand. The accumulated liquid remains in the collection tube while the filter tube and the pestle are discarded.