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Assessment of The Influence of Temperature on Brain DNA Quality for Forensic Studies Using RAPD Marker

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Abstract
Forensic samples may need to be preserved for an extended period, therefore determining an optimal temperature that allows for quality DNA to be obtained when required is essential. This study seeks to ascertain the influence of temperature and postmortem time on the quality of brain DNA for forensic studies. Seven (7) albino Sprague-Dawley mice weighing between 20 – 30 grams were used for this study. One (1) mouse which served as the control was sacrificed and the brain tissue harvested for DNA extraction immediately. The remaining six (6) were divided into two groups, where one group's harvested brain tissues were stored at room temperature of 24℃ and the other group's harvested brain tissues were preserved by freezing at -18℃. DNA was extracted from the brain tissues of one mouse each from the two groups after they had been stored at the different temperatures for 24, 48 and 72 hours. DNA extraction was done using DNA extraction kit from APS Life Tech West Africa Limited and PCR was carried out on each of the DNA extracts using RAPD-OPC-04 primer. DNA concentration and # extracted DNA was determined by spectrophotometry analysis, and the gel electrophoresis was also done to determine the DNA band quality. The results from the spectrophotometry analysis showed that there was consistent decrease in the DNA yield and purity from stored brain tissue samples as compared to the freshly harvested brain sample. The gel electrophoresis showed consistent bands for the DNA extract from the freshly harvested brain tissue and the brain tissue that was preserved at -18℃ for 72 hours. Though, the best nuclear DNA quality is obtained from freshly harvested brain tissues, the quality of nuclear DNA from brain tissue is better preserved by freezing the brain tissue for long periods than at room temperature.

Keywords: DNA quality, Postmortem time, Temperature, Brain, RAPD-OPC-4 primer, Spectrophotometry.

Introduction

Deoxyribonucleic acid (DNA) is a molecule that carries all the information that an organism requires to function and reproduce (Goodwin *et al.*, 2007), and defines the properties of the organism, and are present in every cell and are transferred from parent cells to their daughter cells (Klug *et al*., 2012). The growth in knowledge of DNA and its applications have been used extensively in the fields of proteomics, genomics and forensic biology (Goodwin *et al*., 2007).

Although over 99% of the DNA sequences in the human genome are identical between individuals, a small number of sequence differences are used to distinguish all humans (Saad, 2005). Forensic DNA analysis characteristically deals with materials recovered from crime scenes, paternity testing and the identification of human remains (Jobling and Gill, 2004). DNA analysis remains the key to acquiring information from biological material to aid enquiries associated with criminal offences, disaster victim identification and missing persons' investigations. Both the scope and scale of DNA analysis in forensic science is set to continue expanding for the foreseeable future (Goodwin *et al.*, 2007).

Reliable analysis of degraded DNA is of great importance, since its results impact the quality and reliability of expert testimonies (Maciejewska *et al*., 2013). In forensic science, high quality DNA from challenging biological samples is a key tool for subsequent DNA profiling (Philips *et al.*, 2012). Determining the quality of DNA may provide precise way to estimate the postmortem interval (Liu *et al*., 2001). Therefore, it is important to understand the effect of postmortem interval on DNA degradation of samples obtained from organs (El-Harouny *et al*., 2008). Post-mortem changes prior to sampling are expected and create notable variation in the quality of DNA (Rahikinen *et al.*, 2016). As the relevance and value of DNA profiling to forensic investigations has increased, so too has the desire to generate this information from DNA of smaller amounts and/or affected by diverse environmental conditions (van Oorschot *et al.*, 2010).

Studies have documented the controlled effects of some environmental factors, such as temperature, humidity, ultraviolet radiation and even soil, on the ability to obtain intact DNA for forensic purposes (Dissing *et al.*, 2010). Variables influencing condition of samples such as age of sample (Santini *et al*., 2007), weather conditions (Piggott, 2004) and diet, which may vary among species and within species as diets vary by individual or season (Maudet *et al*., 2004) have also been observed to influence DNA amplification success. Some studies have suggested that success rates will be highest when samples are as fresh as possible and climatic conditions are either dry (Piggott, 2004) or very cold (Lucchini *et al*., 2002).

The need to address questions of preservation often necessitates sampling across large, heterogeneous regions where sample age at time of collection is unknown and conditions are neither dry nor cold (i.e. in the tropics) (Millspaugh and Washburn, 2004). The effects of environmental insults on the ability to recover meaningful DNA profile results may differ drastically from one local environment to another (Barbaro *et al.*, 2008). Brain tissue is more immune to decomposition allowing for the detection and quantitation in this sample when compared with centrally located organs (e.g. liver) and cavity fluid. Thus, brain tissue has some advantages over other specimens collected at autopsy (Rohrig and Hicks, 2015). Therefore, this study is aimed at evaluating the impact of sample storage temperature and at different post-mortem intervals on the quality of the DNA extracted from brain samples.

Materials and Methods

Chemical and Samples

The reagents used in this study were the DNA extraction kit and the RAPD-OPC-04 primer, which were obtained from APS Life Tech West Africa Limited. Seven male Sprague–Dawley albino mice (weighing 20 – 30g) were obtained from the Animal House of National Institute for Medical Research (NIMR), Yaba, Lagos. The animals were kept to acclimatize in the animal room of Cell Biology and Genetics Department, University of Lagos for a week.

Sample Collection

The mice were designated into three (3) groups; three (3) mice in group A and group B while group C had one (1) mouse which served as the control (the zero-hour sample). The mice were sacrificed via cervical dislocation and the brain tissues of the animals were harvested. Group A served as the group with brain tissue samples preserved at room temperature and Group B served as the group with brain tissue samples preserved by freezing. Samples were collected from the two groups for DNA extraction each day at 24 hours, 48 hours and 72 hours.

DNA Extraction

The brain tissue sample of about 25mg were cut, put into microcentrifuge. Micropestle was used to grind the brain tissue sample, 200 µL TG1 buffer was added and well mixed using the micropestle. Proteinase K (10mg/mL) of 20 µL was added to the sample mixture and thoroughly mixed by vortexing. The sample was then incubated at 60℃ overnight for the tissue to be completely lysed. Then 200 µL of TG2 buffer was introduced into the sample mixture and thoroughly mixed by pulse-vortexing. The sample mixture was incubated at 70 ℃ for 10 minutes, and 200 µL ethanol (98 %) was added to the sample mixture, and then mixed thoroughly by pulse-vortexing for 3 minutes.

Place a TG Mini Column in a collection tube then the mixture was carefully transferred to TG Mini Column and centrifuged at full speed for 1 minute. The TG Mini Column was placed in a new collection tube, and 400 µL Wash buffer was added to the sample mixture in the TG Mini Column, then centrifuged at full speed (13,000 rpm) for 1 minute and the flow-through was discarded. The sample mixture in the TG Mini Column was treated with 750 µL Wash buffer and centrifuged at full speed for 1 minute then the flow-through was discarded. The TG Mini Column containing the sample was then centrifuged at full speed for 3 minutes. The sample mixture in the TG Mini Column was treated with 100 µL preheated Elution buffer and allowed to stand for 3 minutes. The mix was centrifuged at full speed for 2 minutes.

Samples are placed in cuvette, after the cuvette had been rinsed with sterilized water and air-dried on a soft absorbing tissue paper. 50µL of the TE buffer was dispensed into the cuvette and 5µL of the sample was also dispensed into the cuvette. The cuvette was then properly placed in the spectrophotometer machine which exposes the samples to UV wavelengths 260nm and 280nm. Readings for the DNA concentration (ng/µL) and DNA purity of each sample was taken at 260 nm and 280 nm.

Table 1: The RAPD Primer and Sequence Used in This Study

Primer	Forward Sequence	Reverse Sequence
RAPD-OPC-04	CCGCATCTAC	GGCGTAGATG

PCR Amplification

The PCR was performed in 25µl of a reaction mixture containing DNA 3µl, master mix 12.5µl, 20 Pico mole primer, and nuclease free water of 7.5µl. Thermocycling was conducted in an Eppendorf microcentrifuge of an initial denaturation of 94°C for 5 minutes, followed by 1 minute at 94°C, 1 minute at 35°C and 1 minute at 72°C following 40 amplification cycles. Then, a final extension step of 10 minutes at 72°C. The samples from the PCR amplification were separated using agarose gel electrophoresis and visualized by ethidium bromide staining.

Statistical Analysis

SPSS software version 20.0 was used to carry out the t-test for the analysis of the DNA extracts. Power value was considered significant at $p<0.05$.

Results

The DNA concentration and purity of the all the DNA extracts from the brain tissues obtained in this study that were preserved at room temperature of 24°C for 24, 48, 72 hours and from the freshly sacrificed animal (labelled C) is shown in Table 2. The highest DNA concentration (95ng/µL) and purity (1.86) was obtained from the DNA extract of the freshly harvested brain tissue. The lowest DNA yield (60ng/µL) was obtained from the DNA extract of the brain tissue that was stored at room temperature for 48 hours while the lowest DNA purity (1.55) was obtained from the DNA extract of the brain tissue stored at room temperature for 24 hours.

Table 2: DNA yield and purity from freshly harvested brain tissue and brain tissues preserved at room temperature (24°C) For 24, 48 And 72 Hours.

Samples	DNA Concentration (ng/µL)	DNA Purity
C (Zero Hour)	95	1.86
A1 (24 Hours)	71	1.55
A2 (48 Hours)	60	1.58
A3 (72 Hours)	70	1.70

The DNA yield and purity of the DNA extracts from brain tissues stored by freezing at -18 ℃ for 24, 48 and 72 hours are shown in Table 3. The highest DNA yield (91ng/µL) from the DNA extracts from brain tissues stored at -18 ℃ was obtained from the brain tissue that was stored for 24 hours while the lowest DNA yield (54ng/µL) was obtained from the brain tissue stored for 48 hours. The highest DNA purity (1.72) from the DNA extracts from brain tissues stored at -18 ℃ was obtained from the brain tissue that was stored for 72 hours while the lowest DNA purity (1.61) was obtained from the brain tissue that was stored for 24 hours.

Table 3: DNA yield and purity from brain tissues preserved by freezing at -18°C for 24, 48 and 72 hours.

Samples	DNA Concentration (ng/µL)	DNA Purity
B1 (24 Hours)	91	1.61
B2 (48 Hours)	54	1.65
B3 (72 Hours)	60	1.72

The mean and standard deviation of the DNA concentration of all the samples are 71.57ng/µL and 15.84ng/µL. The mean DNA yield of the samples stored at room temperature is 67.00ng/µL, which is slightly lower than 68.33ng/µL, the mean DNA yield of the samples preserved by freezing at -18 ℃. The mean and standard deviation of the DNA purity of all the samples is 1.67 and 0.10 respectively. The mean DNA purity of the samples stored at room temperature is 1.61, which is slightly lower than 1.66, the mean DNA purity of the samples preserved by freezing at -18 ℃.

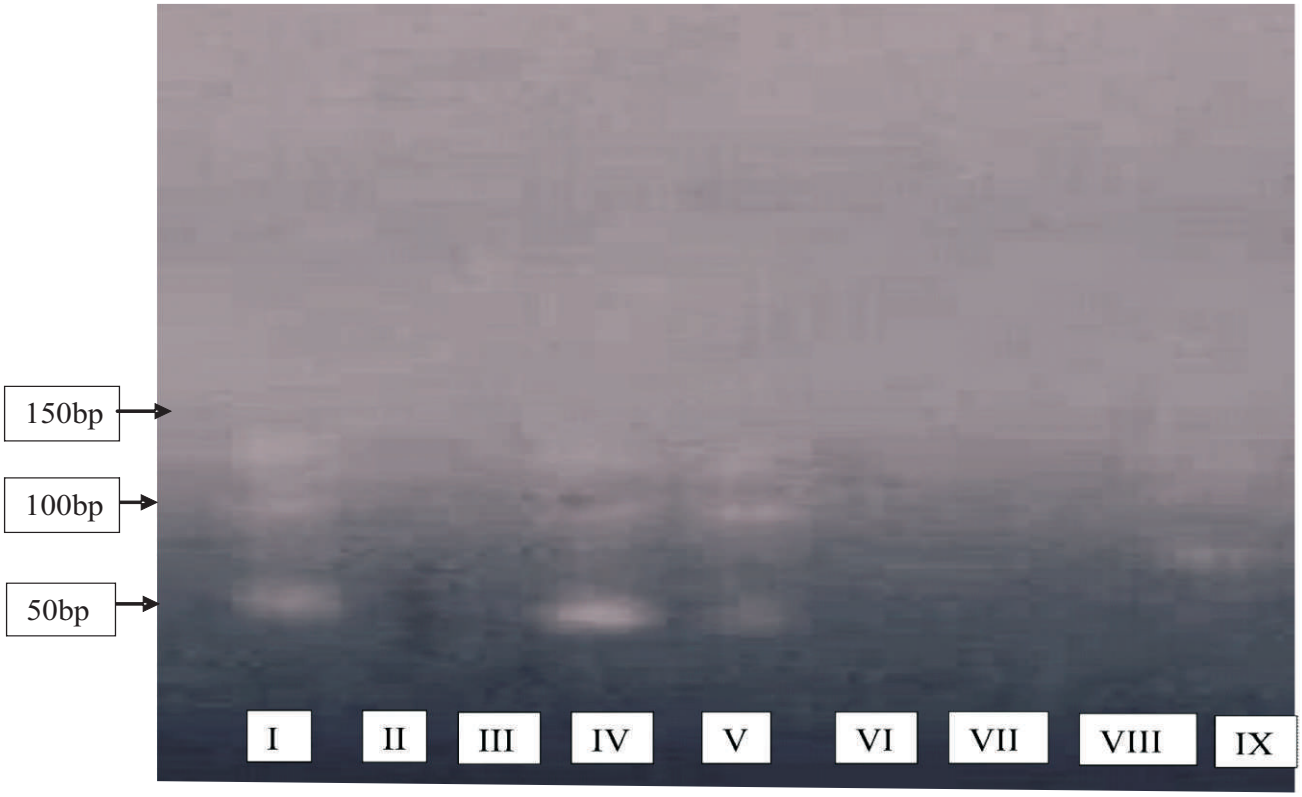

Plate 1: Amplified brain DNA extracts of freshly harvested tissue, tissues stored at 24 ℃ and brain tissues stored at -18 ℃ from albino Sprague-Dawley mice in the study.

I - Ladder.
II - Lane of DNA extract from brain tissue stored at -18℃ for 24 hours.
III - Lane of DNA extract from brain tissue stored at -18℃ for 48 hours.
IV - Lane of DNA extract from brain tissue stored at -18℃ for 72 hours.
V - Lane of DNA extract from freshly harvested brain tissue.
VI - Lane of DNA extract from brain tissue stored at 24℃ for 24 hours.
VII - Lane of DNA extract from brain tissue stored at 24℃ for 48 hours.
VIII - Lane of DNA extract from brain tissue stored at 24℃ for 72 hours.
IX - Ladder.

Discussion

Postmortem time determination is one of the valuable subjects in forensic science (Hao *et al*., 2007). The determination of the quality and quantity of DNA should proffer a more precise way to estimate postmortem interval (Swango *et al*., 2006). There have been previous studies on the different tissues with attempts to estimate the use of such tissues for the estimation of postmortem time (Ebuehi *et al*., 2015). The results of this study showed that the postmortem time and temperature of preservation has immense influence on the quality of the DNA from brain tissues. Brain tissue samples stored at room temperature are more liable to decomposition as the temperature is more suitable for enzymatic activity and bacteria survival.

The result of this study indicates that not only is refrigeration a good means of tissue preservation, but also that the longer a brain tissue is preserved through refrigeration, the better the quality of the nuclear DNA obtainable. This is consistent with the study by Matsuo *et al.* (1999), who stated that the use of cold storage which includes refrigeration is used for tissue preservation, as this reduces the chemical or physical modifications of samples.

In the course of this study, different annealing temperatures (28℃, 30℃ and 35℃) were attempted for the amplification of the brain DNA. The 28℃ and 30℃ annealing temperatures produced no bands for the amplicons on the gel. This finding is contrary to that of Ebuehi *et al*. (2015), who got good bands for DNA amplicons after using 28℃ as the annealing temperature for PCR. But this study is consistent with Ebuehi *et al*. (2015), who stated that decays and decomposition after death in biological samples leads to damage which is manifested in many forms in different tissue samples, as the brain tissue samples stored at room temperature were greatly degraded at 48 and 72 hours.

Brain DNA possesses better quality when extracted from fresh brain sample, the DNA degrades with increase in postmortem time simultaneously with the decomposition of the brain tissues. Though refrigeration is a well-tested means of preservation for forensic samples, it produces better quality of DNA with longer period of preservation. And a warm temperature encourages decomposition of brain samples and DNA degradation and in effect, reduces the quality of DNA obtainable from the brain sample. Therefore, the lower the temperature and the longer the period of storage the better the quality of the brain nuclear DNA.

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