Earphone Swab-Derived 126bp and 143bp mtDNA at D-loop Region: Electrophoretic Band Reaction on Room Temperature

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Abstract: Exploration of crime scene devices, leftover, and its surrounding is of significance in forensic identification due to its potential harbor of biological evidences. Such harboring of evidences proposes traceability under Locard's principle. By body use-contact, devices such as earphones potentiate revelation of the unknown by analysis of cerumen or traces logged from ear. Despite of this usability, environmental challenge is suggested to impact the forensic vitality of the logged evidences. To understand the phenomena, this study applied 30 used earphones at 0, 1, 7, 14 and 20 days room temperature exposure. From the study; amplified electrophoretic reaction showed that, 143bp (HVS I, nt 16268-16410) mtDNA D-loop region reacted with a positive (+) detection only for day 1 (4 [66.67%] samples) and day 7 (3 [50%] samples) of room temperature exposure. Meanwhile, visualization of the results of 126bp (HVS II, nt 34-159) mtDNA D-loop region showed a positive (+) detection only for day 1 (2 [33.37%] of samples) and day 7 (6 [100%] of samples) of room temperature exposure. Data interpretation conclude the effect of room temperature on the trend and quality of earphone swab-derived DNA from day 1 to day 20.

1 BACKGROUND

Forensic identification by means of Deoxyribonucleic Acid (DNA) analysis is modern and referred as among of the three primary independent preferred methods in practice (Dumache et al. 2016; Ruitberg et al. 2001). Its accuracy is by its stability inside biological evidences (International Committee of the Red Cross 2009). Traces of these biological evidences are either freely dispersed at criminal environment or found logged and harbored in objects/devices lastly used and left at the scene (Ah Van Oorschot et al. 2010; Song et al. 2015; Shaler 2011). Such devices harboring samples include earphones under the use-contact theory by Locard's principle (Byard et al. 2016). Logging of the biological samples into earphone attached to the outer ear skin is by expelled cerumen and degrading body cells (Shokry & Filho 2017). In forensic, these devices are becoming useful and crucial in investigative tasks as reported by Seo et al. (2002) though challenged by exposing condition.

Among the challenging condition aggregated by environmental factors, temperature is potentially studied to impact successful profiling hence hindered identification. (Caputo et al. 2011; Toothman et al. 2008). Theory to improve analysis have opted for mini-primer (Butler 2007; Hwan et al. 2008). The method profile reduced size of Short Tandem Repeat (STR) locus (Coble & Butler 2005) also hyper variable segment [HVS] 1 or 2 of mitochondrial DNA (mtDNA), as amplification products (Gabriel et al. 2001). This study, therefore, present the association of room temperature on electrophoretic visualisation as part of the project analysed the effect of room temperature on the quality of earphone swab-derived DNA at 126bp and 143bp.

2 MATERIALS AND METHODS

The laboratory experimental study designed a time serial of exposure of 0, 1, 7, 14 and 20 days interval. Finally, analysis presented the electrophoretic visualization of earphone swab-derived

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mitochondrial DNA resulted from room temperature effect on logged samples.

2.1 Volunteer Consent and Sample Preparation

A total of 30 samples of earphone swab derived DNA was taken from respondents who have agreed and signed a written informed consent. The materials of study were earphone swabs.

2.2 Experimentation protocol

The materials for DNA extraction were DNAzol reagent (Invitrogen, ThermoFisher Scientific, Waltham, MA, USA) and 70% ethanol (EMSURE®, Merck KGaA 64271 Darmstadt, Germany). The material for Polymerase Chain Reactions (PCR) were PCR mix - 12.5 µl (Promega Corporation, Madison, USA) comprising of dNTPs (ATP, CTP, TTP GTP), MgCl2, Taqpolymerase and Nuclease-free water; mtDNA primers - 143bp (HVS I, nt 16268-16410) AFDIL 5'CACTAGGATACCAACAAACC3' and 5'GAGGATGGTCAAGGGAC3' according to Edson et al. (2004), 126bp (HVS II, nt 34-159) AFDIL 5'TCTCCAAGCGGGTGGTGCATTTA3' and 5'AAATAATAGGCAGGAGAGGATATC3' according to Gabriel et al. (2001).

3 RESULTS AND DISCUSSION

Polymerase Chain Reaction Amplification and Electrophoresis Detection

Cell lysed and isolated during extraction produced DNA template for PCR processing. The template under this study was polymerized and amplified using two hyper variable segments: (HVS) II (126bp, nt 34-159) and HVS I (143bp, nt 16268-16410) of D-Loop region of the mtDNA as shown in Figure 1 to Figure 4**.**

143bp and 126bp mtDNA amplicon profiling at day 1 and 7

Profiling of 143bp and 126bp resulted into a band contrast formation. Samples amplification became positively detected on both primers (143bp and 126bp) from day one and seven, though two samples under 126 appeared fainted after 7 days exposure (Figure 1).

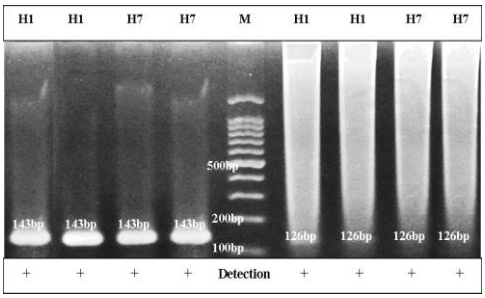

Figure 1: Electrophoretic visualization of 143-bp and 126-bp PCR products of earphone swab samples (M = marker, H1& H7 = samples at day 1 and 7)

143bp mtDNA amplicon electrophoresis of day 1, 7, 14 and 20

Under 2% agarose gel electrophoresis 143bp DNA amplicon, day 1 present positive [+] detection of two samples with fainted bands and other 2 samples demonstrated a negative [–] detection. Day 7, 3 samples detected negatively [–] (no band displayed) and there was only one sample that showed a positive [+] detection with a faint band too. Amplification and electrophoresis of samples by 143bp (HVS I, nt 16268–16410) D-loop mitochondrial DNA amplicon on day 14 and 20 using 2% agarose gel displayed no band or negative detection as shown in Figure 3.

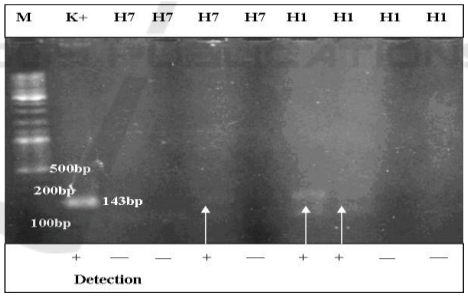

Figure 2: Electrophoretic visualization of 143-bp PCR products of earphone swab samples (M = marker, H1& H7 = samples at day 1 and 7)

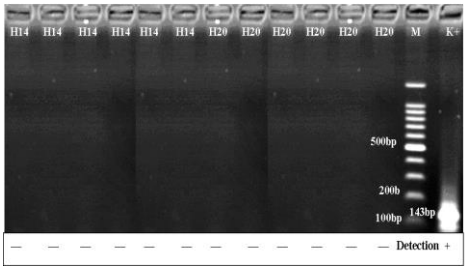

Figure 3: Electrophoretic visualization of 143-bp PCR products of earphone swab samples (M = marker, H14& H20 = samples at day 14 and 20)

126bp mtDNA amplicon electrophoresis of day 7, 14 and 20

Amplification and electrophoretic visualization of 126bp mitochondrial DNA amplicon using 2% agarose gel on PCR swabbed earphone products exposed at 7, 14, and 20 intervals displayed variably. Day 7 exposed sample amplification gave a positive [+] contrast detection, bands were visible for the mtDNA as shown in presented in Figure 4. Contrary to day seven, amplification and electrophoresis of samples exposed for 14 and 20 days resulted into a no display of band contrast hence a negative [–] detection.

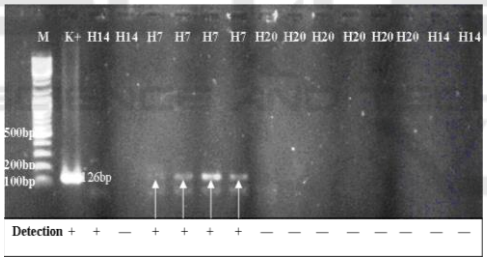

Figure 4: Visualization of 126-bp PCR products of earphone swab samples (M = marker, H7, H14 & H20 = samples at day 7, 14 and 20)

Relative extent effect of exposition on day 1, 7, 14 and 20

A representation of six samples for general percentage effect according to analysis is summarized in Table 1. The results show that, detection of the effects of room temperature exposure on earphone swab-derived 143bp (HVS I, nt 16268–16410) and 126bp (HVS II, nt 34–159) mtDNA D-loop region depend also on the exposure interval and the D-loop specific region between the two (143bp and 126bp). Day one exposition analysis, 66.67% (4) of samples were positively detected at 143bp (HVS I, nt 16268–16410) D-loop region while 33.37% (2) were negatively detected similar to those positively detected at 126bp (HVS II, nt 34–159) D-Loop region. On day 7; 50% (3) of samples were detected at 143bp (HVS I, nt 16268–16410) D-loop region and 100% (6) of samples detected at 126bp (HVS II, nt 34–159) D-loop region. Day 14 and 20, visualized a zero percentage of DNA positive detection due to exposure effect to room temperature on all earphone swab samples (143-bp (HVS I, nt 16268–16410) and 126bp (HVS II, nt 34–159) D-loop region).

Table 1: Results of detection of the effect of room temperature exposure on earphone swab-derived 143-bp (HVS I, nt 16268–16410) and 126-bp (HVS II, nt 34–159) mtDNA D-loop region.

Exposure Interrval	HVS I 143bp nt: 16268 – 16410		HVS II 126bp nt: 34 – 159	
	Detection (+)	Detection (-)	Detection (+)	Detection (-)
Days 1	4(66.7%)	2(33,3%)	2(33,3%)	4(66,7%)
Days 7	3(50%)	3(50%)	6(100%)	0(0%)
Days 14	0(0%)	6(100%)	0(0%)	6(100%)
Days 20	0(0%)	6(100%)	0(0%)	6(100%)

4 DISCUSSION

126bp and 143bp mtDNA Electrophoretic Band Reaction on Room Temperature

According to bands formation, analysis descriptively derived information from detection and contrasting. Detection of effects of room temperature is related to DNA quality contained. DNA quality includes DNA levels, DNA purity and DNA condition state (degradation). DNA degradation is suggested to be the causes of DNA detection failure in PCR-based DNA analysis, this is well portrayed in Figure 3 and some bands in Figure 2 and 4. This is in consistent with (Bartlett & Stirling 2003) with regard to several possible causes for failure detection of DNA, including a minimal amount of target DNA, DNA degradation that prevent primer from annealing, lack of DNA polymerase and PCR cycle also presence of PCR inhibitors. The failure of amplification is characterized by the absence of bands on the

electrophoresis results due to lack of DNA polymerase and PCR cycle. This can be controlled by the use of PCR master mix (Lorenz 2012). From the article lack of cycle in a PCR reaction is controlled by optimizing the primers used for PCR while adhering to Mg^{2+} recommended concentration (should be 1.5 to 4 mM). An excessive amount of taq polymerase in PCR mix causes a smear formation (non-specific reaction) on electrophoresis, whereas inadequacy of it causes a reduced efficiency of amplification and non-appearance of bands.

According (Hall et al. 2014; Schuch et al. 2013), DNA damage caused by external factors such as extreme temperature will result in many types of damage, like damage to DNA strands (both double and single strand), base damage (damage to DNA bases), sugar damage and even presence of DNA crosslink and DNA–protein crosslink – hence DNA degradation. This degradation can be fast or slow, depending on the affecting factors and length of exposure. Findings of this study shows that earphone swabs can still be used as a material for DNA analysis of mtDNA with regard to the effects of exposure to room temperature (28.5°C–30°C) up to day 7. This is in potential consideration when applied to expedite forensic identification, especially in a mass disaster or criminal cases on the crime scene with little trace evidence.

5 CONCLUSION

This study concludes that; the length of exposure to room temperature has effect on the quality of earphone swab-derived DNA. Thereby visualization of the results of PCR on 143bp (HVS I, nt 16268-16410) mtDNA D-loop region showed a positive (+) detection only for day 1 (4 [66.67%] samples) and day 7 (3 [50%] samples) of room temperature exposure. Meanwhile, visualization of the results of PCR on 126bp (HVS II, nt 34-159) mtDNA D-loop region showed a positive (+) detection on similar exposure interval - day 1 (2 [33.37%] samples) and day 7 (6 [100%] samples) of room temperature.

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