70% Ethylated and Non-Ethylated Swab: A Trace Evidence Recovery Method and Usefulness in Spectacles Forensic Evidence

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Abstract: Trace evidence recovery has been studied to involve a number of methods with the inclusion of swabbing methods. In swabbing, different fluids have also been proposed with minimal highlight to ethylated swabbing (prepared from ethanol). Through the use of swabbing fluid at 70% dilution (70% ethylated swab) run simultaneously with distilled water swab, the findings discovered were that 70% ethylated swab was found to concentrate large amount of DNA sample (1421µg/ml), twice the amount recovered by distilled water swab (654.5µg/ml). In terms of purity of recovered DNA; 70% ethylated swab presented nearly similar purity with distilled water DNA pellet (ethylated swab yielded 1.219 purity ratio while distilled water swab yielded 1.176). Electrophoresis DNA molecule migration displayed multiple bands in contrast with 70% ethylated swab from positive to negative electrode. This shows the presence of both small and larger sized DNA fragments. Distilled water swab displayed one deep band near negative terminal electrode. Thereby; the study finding analysis illustrates and suggests that 70% ethylated swab is a useful and strong method in recovering trace DNA evidences for successful DNA profile establishment. In addition, spectacle is prospective a potential harbor of trace DNA sample for forensic investigation.

1 BACKGROUND

Recovery of micro- to macro-Deoxyribonucleic acid (DNA) trace evidence is studied to involve adhesive tape, forceps, and vacuum methods (Fisher et al. 2007; Ah Van Oorschot et al. 2010). Swab method is also discussed and presented to work properly and is far better, while moist in recovering such trace evidences (van Oorschot et al. 2003; Ah Van Oorschot et al. 2010; Jack Dillon, Debra Figarelli, David Sylvester 2009; Oregon State Police 2015; Puritan 2016; Adamowicz et al. 2014). It is opted and used as moisturising fluid in case of remains of high and efficacy determinant. To explore the effectiveness, the experimental studies done have explored the success of ethyl moist swabbing, though most still recommend water moistening option (van Oorschot et al. 2003; Raymond et al. 2008). Thus, in the DNA retrieval study; 95% ethanol swab (Slrichantrawonq et al. n.d.) was found to be suitable to recover DNA at a high yield than distilled water (Hildebrand et al. 2004), in which a 25% ethylated alcohol swab yielded the most compared to those with 50% concentration and distilled water swab. Emphasizing essence to these innovations is the maximized retention of minute natured sample residing on swab after recovery against loss or blow-away (Fisher et al. 2007). Contrary to a noted good use as swab fluid, ethyl on the other hand is presented as a decontaminant and lyses catalyst (Gršković et al. 2013), which means that it has a destructive effect in opposition to recovery usefulness. Despite of substantial trials on alcohol fluid, specified ethyl percentage which is effective to be used in swabbing is yet to be established. This study therefore dedicated furthered investigation on a 70% ethylated swab supply on spectacle evidence convinced by reasons below;

1.1 Why 70% Ethylated Alcohol Swab?

The percentage of alcohol on treatment of biological sample has a direct relationship. On DNA extraction and preservation usage, 70% ethyl has evidenced a

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flexible treatment of biological samples which allow morphological exploration afterwards (Oswald 2007). This is in contrast to high or low percentage in respect to a volatile, drying blow-away, non-flexible, and degradation-prone performance. In addition; compared to other alcohol groups such as isopropanol, ethanol is highly precipitous that resuspend DNA pellet easily. The insolubility of DNA molecules is catalysed by forming H-bonds with water during isolation (decrease hydration ability of water to DNA) – reduced decaying. Lower dielectric leads DNA to aggregate and concentrate with cations below lighter molecules under phenol-chloroform extraction (Brennan 2017; Zumbo 2013).

Referring to the percentage used in the reference above, ethylated alcohol swab envision has a special use in recovering DNA trace evidence. 70% ethyl alcohol swab is optimised for consideration as explained earlier. This ground prompted the exploration of the usefulness of 70% ethylated swab through admission of spectacle as useful and reliable suggested source of trace biological evidence based on Locard's and Kick's contact and silent witness respective principles. Appreciation of 70% ethyl swab and admission of spectacle, in addition to normally referred evidences such as clothes, knife, vehicles, firearms, bedding, food, condoms, lip cosmetics, wallets, jewellery, glass, skin, bullet, paper, cables, windows and door lockers/handle, stones and watch (Ah Van Oorschot et al. 2010) broaden exhibits. Either in recovery of such micro or macro exhibits like hair, dust, soil, glass particles, fluids, touched surfaces, clothes (Fisher et al. 2007) as forensic evidence, limited information is on the spectacles as potential source of biological trace evidence. Apart from compilation through literature reviews of the established useful properties of the ethyl alcohol in forensic evidence; this study complemented the findings used to recover trace DNA from spectacles especially through the use of 70% saturation. Aggregation of these information potentiate establishing special 70% ethyl swab for trace evidences swabbing recovery as reported in this study compared to most recommended water swabs.

2 MATERIALS AND METHODS

The article paralleled literature reviews on available studies of ethylated alcohol swab application or usefulness and experimental authentication of spectacle evidence. Experimental content was conducted at the University Human Genetic Laboratory involving two biological samples swabbed from two different spectacles: one by a 70% ethyl swab and the other by a distilled water swab.

2.1 Sample Recovery

Samples to determine the usefulness and application of ethylated swab was obtained from two participants who voluntarily gave their spectacles after being given a clear understanding of the study purpose. From two spectacle evidences, DNA trace biological sample was recovered by a separate swabbing under one swabbing direction and surface without repetition. The two swabs used were sterilised and cotton-made. One swab was a readymade 70% ethylated which swabbed one spectacle, and the other was a dry swab which was moistened by 1cc of distilled water and swabbed the second spectacle. The process was immediately followed by a tube soaking of spectacle swabbed swabs into 2 different tubes filled by 4cc distilled water overnight to allow down settling of DNA biological traces recovered for DNA analysis.

2.2 DNA Extraction

The extraction process proceeded with removing of upper most fluids while retaining down settled sample solution. Then, 0.5cc of each sample was isolated in a sterile centrifuge tube; pipetted with 1cc of DNAZol (Invitrogen, ThermoFisher Scientific, Waltham, MA, USA), and vortexed and incubated for 15 minutes. Then, it was vortexed with 0.2cc of Chloroform (Merck KGaA, 64271 Darmstadt, Germany) followed with a centrifugation at 8,000 rpm for 10 minutes. Separated supernatant was obtained into eppendorf with isopropanol 1cc (EMSURE®, Merck KGaA, 64271 Darmstadt, Germany), 15 minutes incubation, and centrifugation at 12,000 rpm for 10 minutes then with care followed by the discarding of supernatant fluid again, leaving settled and concentrated pellet. The pellet was washed with 0.5cc of 70% ethanol (EMSURE®, Merck KGaA 64271 Darmstadt, Germany), and it then underwent 15 minutes of centrifugation at 12,000 rpm for 5 minutes, which again was followed by the removal of supernatant through Chen et al. (2010) as well as Chomczynski et al. (1997) protocols. Finally, 50µl of distilled water resuspended formed DNA pellet for spectrophotometer and electrophoresis.

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2.3 Spectrophotometer Measurements

Spectrophotometry measurement was done in order to establish concentration and quality parameters of 70% ethylated swab in reference to distilled water swab, as well as the potential extent of spectacle evidence in yielding significant biological sample for DNA. Using Ultraviolet-visible Spectrophotometer (UV-1601, PC, Shimadzu, Japan), DNA Concentration was determined by absorbance reading at 260nm and 280 in UV-1061, while DNA Purity was given by Optical Density (OD) OD260/OD280 ratio, refer Table 1.

2.4 Electrophoresis

Electrophoresis of acrylamide gel method was opted due to sensitivity even to minute sample without Polymerase Chain Reaction (PCR) (purposely to find out if both 70% ethylated swab and spectacle referenced to water swab can yield interesting results without polymerase amplification). The gel was prepared by a 3cc acrylamide reagent (Sigma-Aldrich) mixed with 8cc Tris-borate-EDTA (TBE) - 0.5x (Promega Corporation, Madison, USA). Then, Temed (Sigma-Aldrich) 20µl followed with 200µl ammonium perisulfate solution (Sigma-Aldrich) under homogenization cycles at 100 Volts for 60 minutes (Figure 3).

3 RESULTS AND DISCUSSION

3.1 70% Ethyl swab recovery method

According to literature, alcoholic swab has exemplified usefulness in varied percentages. As found from this study, the alcoholic swab with a concentration of 70% was reasonably examined and presented to substitute and establish a useful ethylated swab potential for maximizing recovery of trace biological evidence. Compared the two swabs used, the 70% ethyl alcoholic swab and distilled water swab, the findings presented closer results in spectrophotometer measurement (Table 1) but quite different in electrophoresis band contrast (Figure 3). Concentration reading was measured almost three times in 70% ethylated swab compared to distilled water swab as per Figure 1. This concentration gives the interpretation that 70% ethylated swab recovers more DNA samples compared to possible amount able to be recovered by distilled water swab. In forensic profiling analysis, this interpretation gave a meaning to the usefulness of increased probability and assured the recovery of an adequate amount of sample from targeted evidence of traces that is potential to enable successful profiling results during experimentation.

Table 1: Concentration and Purity of Spectacle DNA evidence under ethylated and non-ethylated swab

Sample Code	Absorbance 260 nm	Absorbance 280 nm	DNA Concentration (ng/ul)	DNA Purity
Distilled Water Swab-A	0.187	0.159	654.5	1.176
70% Ethylated Swab-B	0.406	0.333	1421	1.219

In deducing the purity measurement, the generated purity increased confidence for usage of ethylated swab. Estimated chances of increased degradation and destruction of genetic materials as anticipated through previous few reported applications in decontamination pose a contrary scenario. According to the studies; ethyl being as destructive agent forecasted expectation that this study also significantly generated a lowered purity by the fact of its destructive ability (micro-organisms discussed similar to structure of traces). Purity of the 70% ethylated swab recovered sample was nearly similar above distilled water swab. Despite the fact that both 70% ethylated swab and distilled water swab were below the recommended limits of purity (1.6-2.0) (Table 1), the findings suggested that use of modern extraction would purify to acceptable limits. The nature of results measured project useful pellet (of acceptable limit) with agreed contribution of effective recovery of ethyl swab.

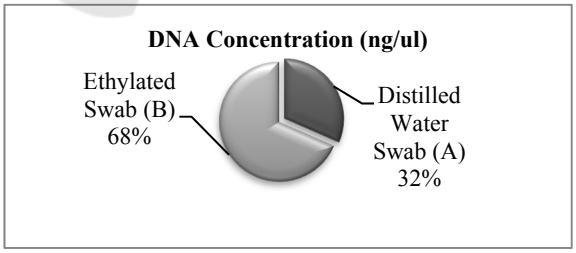

Figure 1: Concentration of DNA extracted from 70% ethyl and distilled water swabbed spectacle.

DNA was recovered with ethyl swab concentrated DNA amount more than twice as high (1421µg/ml) as those concentrated by distilled water swab (654.5µg/ml). The ethyl method concentrated DNA amount due to its ability to recover even the stickiest sample traces as much as possible.

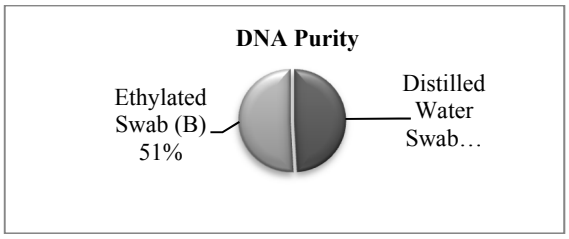

Figure 2: Purity of DNA extracted from 70% ethyl and distilled water swabbed spectacle.

Purity was assessed and compared to sample extracted by water swab/ 70% ethyl swab was measured to have pure DNA in a similar ratio. Purity of DNA was well-detected outside of quality range. Thus, this similar quality (though a bit high in ethyl) complemented the significance of the use of 70% ethylated swab.

3.2 Electrophoretic Reaction

Electrophoresis DNA band contrast appeared to both swabs. Migration of DNA fragments from negatively charged electrodes to positive was established in different numerous band level especially to sample of the 70% ethyl swab as presented in Figure 3. This band contrast portrayed the size, length and strength of the DNA extracted from these two kinds of swabs (ethylated and water swabs). As discussed in concentration and purity above (Table 1), DNA obtained through 70% ethylated swab was with nearly similar purity but higher collected amount as portrayed in Figure 1. This information implis that both swabs yielded pure DNA capable to be analyzed in electrophoresis, as shown in Figure 2. The meaning is that DNA has successfully been electrophorised as full charged fragments and migrated to appropriate contrast level. However, from the displayed bands, ethylated swab contrasted more bands compared to water swab. This suggests that 70% ethyl swab recovered more amount of DNA sample with various strength and size leading to a differed migration of which small-sized fragments were lighter and migrated faster with contrast level closer to Anode electrode as referred in Figure 3. The longer and larger sized DNA fragments recovered in ethylated swab and water swab appeared to contrast closer to cathode electrode due to slower migration of charged fragments.

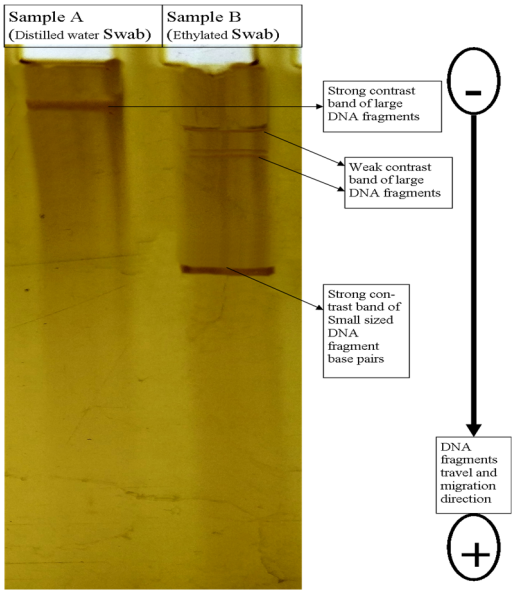

Figure 3: Electrophoresis band contrast of DNA sample recovered through 70% ethyl and distilled water swabbed spectacle evidences.

This interpretation suggests three things. Firstly, both swabs (70% ethylated and distilled water) recovered a significant amount, but ethylated swab recovered more significant minute traces evidenced by different band contrasts and even being concentrated more at lower level (closer to positive end). Secondly the purity of DNA sample recovered by ethylated swab was higher with excess concentration compared to water swab. Thirdly, minute and increased extraction of sample was of useful quality as being able to be profiled on electrophoresis even without PCR primer amplification.

3.3 Spectacle

As other evidences were found at crime scene through this study, spectacle was evaluated to useful and potential evidence able to be used as source of trace DNA for profiling as a result of contact from humans that used it before. Through a well-established recovery method, spectacle exhibit can significantly contribute to the logged and harbored amount of DNA in contacted sample from specific individuals used before.

4 CONCLUSION

DNA spectrophotometer and band visualization contrast depicted and suggested 70% ethylated swab

to be a useful and strong method that recovers large and enough samples for DNA profile establishment. As for the reasons stated above in signifying conduction of this study, 70% of ethyl is in the manner recommended due to it being a flexible percent that tolerates further morphological treatment of DNA sample from recovery and let them in for a temporal storage before processing in the laboratory. The study has also brought attention to the consideration of spectacle as potential source of DNA sample either found at crime scene for criminal linkages or to purposed forensic inquiry for investigative profiling.

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