ORIGINAL ARTICLE

Quantitative Analysis of Nucleic Acids in Sweat with Advantage to Latent Finger Prints

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Abstract

Objectives: Fingerprints are one of the most important evidence of crime scene. In an attempt to recover DNA from latent fingerprint sweat components, DNA extraction is a crucial step in the recovery of DNA due to the fact that latent fingerprints contain low amounts of DNA[7,8] and simple, sensitive techniques are therefore required. **Materials and Methods:** This study was carried out to evaluate the accuracy of 3 different methods employed for DNA quantification, i.e., ultra violet (UV) spectrophotometry, NanoDrop, and Qubit Fluorometry, and to compare the effectiveness of 2 different methods used for DNA extraction, i.e., Proteinase K/phenol/glycogen (PPG) method (developed from the classical method proteinase K/phenol) and commercially available QIAmp DNA mini kit. **Results:** The results showed that Qubit® Fluorometer showed higher accuracy than other two methods and was observed to have the detection limit of 1 ng/μL and the sample volume of 1 μL was sufficient for the detection. Meanwhile, UV spectrophotometry and NanoDrop were found to have the detection limit of 60 and 10 ng/μL, respectively. The results indicated that Qubit® Fluorometer was suitable for detecting low amounts of DNA. The PPG method and QIAmp DNA mini kit were compared for their effectiveness in extracting DNA standards and DNA from fingerprint sweat on A4 papers and it was found that developed method could yield higher amount of DNA than QIAmp kit, which was about 4-fold with DNA standards and about 2-fold with fingerprint sweat. **Conclusions:** The findings indicated that the developed method was far more effective than QIAmp kit and should be considered for use in forensic aspects.

Key words: DNA extraction, fingerprint sweat, Qubit® Fluorometer

INTRODUCTION

Fingerprints are one of the most important evidence of crime scene.[1] Fingerprints found at crime scenes can be classified as patent, latent, and plastic impressions.[2] Patent and plastic prints are noticeable to the human eye without any particular treatment where the contrast between the fingerprint and its background is sufficient for viewing. On the other hand, latent fingerprints are present but invisible without further processing.[2] Latent fingerprints are the most common type of evidence found at crime scenes, and the most problematic since they required methods to develop fingerprints that can be visualized or recorded.[3] For latent fingerprint detection, eccrine (sweat) and sebaceous glands are the most important glands that are responsible for skin secretions within the dermis.[4,5] Eccrine glands are found on the palms of the hands, and various amino acids are present in secretions from these glands, where the extract composition depends upon the individual and a variety of other factors including general health, diet, gender, and age.[6] According to this, recovering DNA from latent fingerprint sweat components is gaining much attention in forensic science since it can provide more complete information than topological patterns.[4] In an attempt to recover DNA from latent fingerprint sweat components, DNA extraction is a crucial step in the recovery of DNA due to the fact that latent fingerprints contain low amounts of DNA[7,8] and simple, sensitive techniques are therefore required. Apart from the extraction step, methods used for DNA quantification are also crucial.

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In this paper,[3] different methods (ultra violet [UV]-vis spectroscopy, NanoDrop, and Qubit fluorometry) employed for DNA quantification were compared in terms of their accuracy and a simple method for extraction (proteinase K/phenol/glycogen method [PPG]) of low amounts of DNA from latent fingerprint sweat was developed and compared to commercially available QIAmp DNA mini kit.

MATERIALS AND METHODS

DNA was quantified in duplicate or triplicate using 3 different methods, i.e., UV-vis spectrometry, NanoDrop, and Qubit® Fluorometer. Quantification was performed according to the manufacturer's instructions using the DNA standards of 0.001, 0.01, 0.1, 1, 10, 25, 50, 100, and 200 ng/μL. DNA extraction from DNA standards DNA was extracted from triplicate samples using 2 different methods, i.e., PPG method (PPG method, developed from the classical method proteinase K/phenol) and commercially available QIAmp DNA mini kit. For both methods, 10 ng DNA standards was applied on A4 papers and was left at ambient temperature to dryness, and DNA was extracted from the dried papers and quantified using commercially available In vitrogen Qubit Fluorometer. DNA extraction from latent fingerprints on A4 papers. The participants were asked to apply their hands on the neck and head for an hour before placing their hands damped with sweat on A4 papers. The papers were allowed to dry at ambient temperature and DNA was extracted from the dried papers using PPG method and QIAmp DNA mini kit and quantified using Invitrogen Qubit Fluorometer. Statistical analyses on the total DNA yields were performed in SPSS version 17.0. The statistical differences were analyzed by one-way analysis of variance. $P < 0.05$ was considered to indicate the statistical difference.

RESULTS AND DISCUSSION

DNA quantification using 3 different methods. It is well-recognized that latent fingerprints left at a crime scene generally deposit lipid and sweat, as well as a certain amount of cells. In this view, sweat is considered as another important fingerprint deposit for the recovery of DNA from latent fingerprints. However, certain measurements should be considered to yield sufficient amounts of DNA due to the fact that sweat contains a very low amount of DNA. Given the importance of approaches required to yield adequate amounts of DNA, we compare the effectiveness of three approaches: UV Spectrophotometry, NanoDrop method and Qubit® Fluorometer. It was observed that using UV Spectrophotometry the DNA concentrations of 60-200 ng/μL allowed for a quick and accurate reading of DNA concentration with an accuracy close to 100%, as presented in Table 1. Meanwhile, the DNA concentrations of 25 and 50 ng/μL lowered the accuracy to 53 and 67%, respectively. Notably, at the concentrations below 25 ng/μL the DNA concentration could not be read. Our results indicate that UV spectrophotometry has the limit of detection of 25 ng/μL and its accuracy and precision could be achieved at the lowest concentration of 60 ng/μL. Using NanoDrop method, an accuracy of 80-100% was achieved when the DNA concentrations were 10-200 ng/μL, as presented in Table 2. Decreasing the DNA concentration to 1 ng/μL lowered the accuracy to 13% and the concentrations below 1 ng/μL could not be read, thereby indicating that the NanoDrop method has the limit of detection of 1 ng/μL and an accuracy reading could be achieved at the lowest concentration of 10 ng/μL. Measured by Qubit® Fluorometer, DNA content could be quantified at the concentrations of 1-100 ng/μL with an accuracy of 80-100% while DNA could not be detected at 200 and 0.1-0.001 ng/μL, as shown in Table 3.

Our findings indicate that Qubit® Fluorometer has high sensitivity and accuracy at the lowest DNA concentration of 1 ng/μL, showing an accuracy of 84%. Taking into account the sensitivity and accuracy of the three approaches, it was clear that Qubit® Fluorometer showed the highest sensitivity, compared to UV spectrophotometry and the NanoDrop method. However, it is worth to note that apart from the DNA concentration applied, sensitivity and accuracy of available approaches are considerably dependent upon the volume of DNA suspension measured; increasing the volume of DNA increases the sensitivity of measurement approaches.

In forensic DNA analysis however, increment of DNA volume is generally avoided since DNA extracted from latent fingerprints has relatively low content. Based on our results, Qubit® Fluorometer was chosen for subsequent studies.

Table 1: Percent accuracy of a UV Spectrophotometer employed for detection of genomic DNA at various concentrations ranging from 0.001 to 200 ng/μL

DNA applied (ng/μL)	DNA detected (ng/μL)[a]	% Accuracy
200	213.33	93.33
100	138.33	138.33
90	93.33	103.70
80	88.33	110.42
70	76.67	109.52
60	68.33	113.89
50	33.33	66.67
25	13.33	53.33
10	−3.33	−33.33
1	−61.67	−6166.67
0.1	−85.00	−85000.00
0.01	−63.33	−63333.33
0.001	−23.33	−2333333.33

[a]N=3

Table 2: Percent accuracy of a NanoDrop® employed for detection of genomic DNA at various concentrations ranging from 0.001 to 200 ng/μL

DNA applied (ng/μL)	DNA detected (1st trial) (ng/μL)[a]	% Accuracy	DNA detected (2nd trial) (ng/μL)[a]	% Accuracy
0.001	ND	ND	ND	ND
0.01	ND	ND	ND	ND
0.1	ND	ND	ND	ND
1	0.13±0.42	13.33	0.13±0.06	13.33
10	8.97±0.42	89.67	8.97±0.42	89.67
12.5	12.43±0.38	99.47	9.50±0.61	76.00
25	21.50±0.38	86.00	20.67±0.76	82.67
50	48.83±0.77	97.67	53.67±2.08	107.33
100	93.17±0.58	93.17	90.33±1.04	90.33
200	185.33±12.33	92.67	-	-

[a]N=3. ND: Not detected

Table 3: Percent accuracy of a Qubit® Fluorometer employed for detection of genomic DNA at various concentrations ranging from 0.001 to 200 ng/μL.

DNA applied (ng/μL)	QF value (N=3)	DNA detected (ng/μL)	% Accuracy
200	ND	ND	ND
100	435.67±2.08	87.13	87.13
50	178.67±0.58	35.73	71.47
25	96.27±0.15	19.25	77.01
10	41.50±0.26	8.30	83.00
1	4.18±0.43	0.84	83.60
0.1	ND	ND	ND
0.01	ND	ND	ND
0.001	ND	ND	ND

ND: Not detected

Qubit® Fluorometer is widely used for DNA quantification in many previous studies due to its accuracy and sensitivity.[9-13] Total DNA yields from DNA standards using 2 different DNA extraction methods in forensic DNA analysis, QIAmp DNA kit is widely employed for DNA extraction since it allows for quick and easy extraction. However, we hypothesized that the kit may yield low amounts of DNA due to the fact that DNA is usually lost during the DNA-membrane binding process, washing and DNA elution. It is well-known that latent fingerprints deposits low amounts of DNA. According to this, processes that can recover high amounts of DNA should be considered. In this study, different extraction processes were compared including QIAmp DNA kit and PPG method. As presented in Table 4, it was noted that DNA extraction using QIAmp DNA kit could yield only 1.48 ng from the initial DNA amount of 10 ng, corresponding to 14.80% of DNA recovery. Meanwhile, the PPG method yielded 6.17 ng of DNA corresponding to 61.73% recovery, which was 4.2-fold greater than that extracted using QIAmp DNA kit. Our results indicate that the PPG method developed in the current study is far more effective than QIAmp DNA kit for extracting DNA from latent fingerprints.

Total DNA yields from latent fingerprints on A4 papers using 2 different DNA extraction methods.

Both QIAmp DNA kit and the PPG method were employed for DNA extraction from latent fingerprints on papers. Again, as shown in Table 5, it was observed that the PPG method was far more effective than QIAmp DNA kit, which yielded 4.23 ng of genomic DNA while only 1.87 ng was yielded by QIAmp DNA kit. No DNA was detected in the control papers. Our findings suggest that the PPG method developed in our study is useful for extracting DNA from latent fingerprints. However, it is worth to note that the amounts of DNA recovered from latent fingerprints depend considerably on several factors such as amount of cells deposited on latent fingerprints, time spent on collection before extraction, contamination of samples with DNA-extraction deterring substances, and deterioration of DNA due to humidity, light, microbes, and pH. Further studies should focus on such factors.

CONCLUDING REMARKS

This research has highlighted the effectiveness of the PPG method for DNA extraction over commercially available QIAmp DNA mini kit, and has also compared the accuracy of DNA quantification using 3 different methods, i.e., UV spectrophotometry, NanoDrop, and Qubit® Fluorometer. Our results demonstrated that Qubit® Fluorometer was far more accurate for DNA quantification than other two methods. Qubit® Fluorometer was observed to have the detection limit of 1 ng/μL and the sample volume of 1 μL is sufficient for detection. On the other hand, UV Spectrophotometry

Table 4: Comparison of DNA contents obtained via two different extraction processes: QIAmp DNA kit and PPG method

Extraction process	QF value (N=3)	DNA concentration given (ng/µL)	DNA content extracted (ng)	% Recovery
QIAmp DNA kit	7.42±0.15	0.074	1.48	14.83
PPG method	30.87±0.35	0.309	6.17	61.73

Table 5: Comparison of DNA contents obtained from latent fingerprints on A4 paper via two 296 different extraction processes: QIAmp DNA kit and PPG method

Sample	QF value (N=3)	DNA concentration extracted (ng/µL)	DNA content extracted (ng)
QIAmp DNA kit			
Latent fingerprint	9.37±0.30	0.09	1.87
Clean paper	ND	ND	ND
PPG method			
Latent fingerprint	21.13±0.57	0.21	4.23
Clean paper	ND	ND	ND

and NanoDrop had the detection limit of 60 and 10 ng/µL, respectively. Our findings suggest that Qubit® Fluorometer is suitable for detection of low amounts of DNA. The developed method (PPG method) and QIAmp DNA mini kit were compared for their effectiveness in extracting DNA standards and DNA from fingerprint sweat, and it was found that developed method could yield 6.17 ng of DNA (from the initial amount of 10 ng) corresponding to 61.73% recovery, which was about 4-fold greater than QIAmp kit that yielded only 1.48 g of DNA corresponding to 14.83% recovery. Our findings indicated that the developed method is far more effective than QIAmp kit and should be considered for extracting very low amounts of DNA.

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