

Evaluation the Suitability of Saliva for DNA Profiling Analysis in Comparative with Blood Samples

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Abstract

Collection, preservation and analysis of body fluids are important aspect of forensic science. Isolated DNA from saliva has become an attractive alternative to the use of blood-derived DNA in performance characteristics. The objectives of this study were to determine the suitability of saliva for DNA profiling analysis and compare blood and saliva as biological source in the performance characteristics. Saliva and buccal swab (as the reference source of genomic DNA) samples were collected from 7 healthy volunteers. The saliva samples were taken with different volumes (100, 200, 300, 400) μl of whole saliva. On the other hand, blood and saliva samples were collected from 25 healthy volunteers in a comparative study. Extraction of DNA was done by Phenol Chloroform method. The results showed difference in the mean DNA concentration which was quantified by using Real-Time PCR from various volumes of saliva samples (100, 200, 300, 400) μl recording (1.23, 2.21, 3.40, 8.24) ng / μl respectively. The mean allele's percentage of 15 STR loci that appeared in profile of different volumes mentioned above were (73.3, 83.7, 89.5, 99.04)% respectively. The mean DNA concentration and the purity from saliva and blood samples were measured by using nonadrop spectrophotometer recording (10.736, 51.164) ng/ μl (1.64, 1.72) respectively. It is concluded that, saliva samples can be taken at different volumes in the field of criminal research instead of the traditional a way of taking blood samples.

Keywords: saliva, blood, genomic DNA, STR.

Introduction

Recent studies revealed that high quality and quantity DNA can be obtained from saliva samples [1]. There has been increased interest in diagnosis based on saliva analyses [2]. The exfoliated buccal epithelial cells in saliva very promising alternative source of DNA because they can be obtained using self-administered, relatively inexpensive techniques, it has a unique fluid and considered a good medium for diagnostic that advanced exponentially in the last 10 years [3].

The traditional source of genomic DNA is blood, but recently saliva has increasingly been investigated as a source of DNA deriving from oral cells [4]. It was used in diagnosis of various diseases; healthy adult persons normally produce 500–1500 ml of saliva per day, at a rate of approximately 0.5 ml/min [5]. The mean number of epithelial cells per 1 ml of saliva is about 4.3×10^5 cell. Moreover, the turnover of epithelial cells is quite extensive in the mouth as the surface layer of epithelial cells is replaced, on average, every 2.7 h [6]. It provides a useful source for biomarker profiling and forensic identification [4]. In

addition, it can be deposited on human skin through biting, sucking, licking, and kissing, etc [7], as well as it can be found on victims of several violent crimes and has been shown to be potentially recovered and typed from bite marks, cigarette butts, postage stamps, envelopes, edibles, and other objects[3].

Blood samples have proven to be a standard source of genomic DNA for biomarker genotyping and in forensic science. However, the need to have a health professional draw the blood as well as the invasive character of this method significantly reduces participation rates [8].

Some study subjects such as psychiatric patients may be reluctant to provide blood samples [9]. Therefore, the DNA that isolated from saliva has become an attractive alternative to the use of blood-derived DNA in genetic studies and is now extensively used in many applications [10]. Short tandem repeat (STR) polymorphisms have been firmly established as standard DNA marker systems since more than 15 years both in forensic stain typing and in paternity and kinship testing [11].

STR are DNA markers, also called microsatellites or simple sequence repeats (SSRs) [12], since in DNA is a class of polymorphism that occurs when a pattern of two or more nucleotides are repeated and the repeated sequences are directly adjacent to each other. The pattern can range in length from 2 to 10 base pairs (bp) [13].

Material and Methods

Sample collection

Saliva can be easily collected from humans, the method used in this study was the “drool” method, where the volunteer simply drools into the tube, the importance of the exact timing of the samples to exclude brushing teeth before the collection to avoid food and fluid ingestion or chewing gum for at least 30 min before collection, and to rinse the mouth with water (preferably distilled) [14]. Saliva and buccal swab (reference sample) were collected from 7 volunteers, saliva samples were taken with different volumes from each volunteer including (100, 200, 300, 400) μl were used for DNA extraction. The volunteers were asked to rinse their mouth with tap water, 30 s before sampling of buccal swabs, to avoid the contamination with food particles. For each individual, both sides of buccal mucosa were swept with a cotton swab for 15s. In addition, blood (fresh whole blood) samples and saliva were collected from 25 volunteers as a comparative study.

DNA extraction: All samples were extracted using organic phenol-chlorophorm method, saliva according to Anzai *et al.*, 2005 with modification [15], blood and buccal swab sample according to Souvik *et al.*, 2013 [16]. Moreover extracted saliva DNA samples from different volume (100, 200, 300, 400) μl for each volunteer were loaded on a 1% agarose gel and visualized by ethidium bromide staining.

Real- Time PCR Amplification: Twenty eight saliva samples from different volumes were measured using multiplex Real-time PCR assay, in addition, the concentration of DNA buccal swab was determined as the reference sample. Amplification reactions were performed on a 7500 fast Real-Time PCR

System and the data were analyzed with the 7500 fast System SDS software v2.0.5 (Applied Biosystems, Foster City, CA). The specific gene detection (Taq man technique) with the commercial kit (Quantifiler human DNA quantification Kit) in 7500 fast Real time PCR system. A multiplexed TaqMan[®] was assembled that amplifies SRY (FAM[™]-labeled probe), RPPH1 (VIC[®]-labeled probe) and an Internal Positive Control-IPC (NED[™]-labeled probe). Assays were designed using the TaqMan[®] Gene Expression (14). Amplification reactions contained 2 μl of standards dilution series (Std) starting concentration from 50 ng/ μl (Std1) to 0.023 ng/ μl or 23 pg/ μl (Std8). Real-Time PCR reaction mix composed of 10.5 μl of Quantifiler Duo Primer Mix, 12.5 μl of Quantifiler Duo Reactino Mix, and 2.0 μl of DNA sample. Real time PCR conditions for amplification were 60°C for 2min, 95°C for 10min, 95°C for 15 s and 60°C for 1min for 28 cycles [17].

Amplification for STRs:

Fifteen autosomal STR markers or loci were genotyped along with the amelogenin locus on the X and Y chromosomes using the Applied Biosystems AmpFISTR[®] Identifiler[™] kit which amplifies the loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, VWA, TPOX, D18S51, D5S818, FGA). Approximately 1 ng of template DNA was amplified for each sample following the protocols described in the Identifiler[™] User's Manual (Applied Biosystems) [21]. The samples were amplified with an Applied Biosystems Veriti[®] PCR System (Applied Biosystems). The PCR conditions for amplification of STR marker were 95°C for 11min, 94°C for 1min, 59°C for 1min, 72°C for 1min and 60°C for 6min for 28 cycles [18].

DNA Typing: Amplification products were diluted 1:15 in Hi- Di[™] formamide and GS500-LIZ internal size standard (Applied Biosystems) and analyzed on a 16-capillary ABI Prism[®] 3130 XL Genetic Analyzer. POP[™]-4 (Applied Biosystems) was utilized for higher resolution separations on a 36 cm array [19].

Data collection: Data collection was performed with Data Collection ver. 2.0 software (Applied Biosystems) and samples were analyzed with Gene-Mapper ver. 3.2 software (Applied Biosystems).

Quantification of DNA: The concentration of 1 μ l DNA sample (saliva and blood) was determined using the Nano Drop ND-1000 spectrophotometer (Nano Drop) Technologies. The 260/280 ratios was calculated by the Nano Drop spectrophotometer and used to evaluate the DNA purity.

Results and Discussion

The yield of extracted saliva DNA samples from the different volumes were evaluated using the gel electrophoresis Fig.(1).

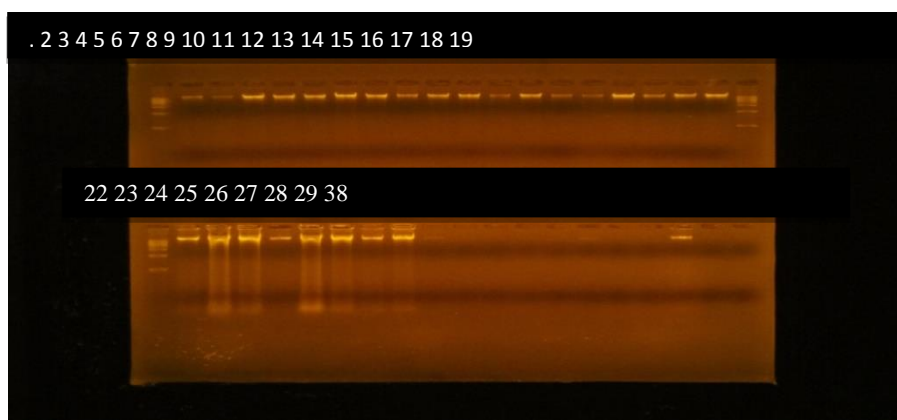


Fig.(1): Electrophoresis of genomic DNA from saliva sample, the gel show The Lane (2, 3, 4, 5) sample No.1, Lane (6, 7, 8, 9) sample No.2, Lane (10, 11, 12, 13) sample No.3, Lane (14, 15, 16, 17) sample No.4, Lane (18, 19, 38) sample No.5, Lane (22, 23, 24, 25) sample No.6, Lane (26, 27, 28, 29) sample No.7, representing four different volumes of DNA sample (100,200,300,400) μ l respectively for each 7 donor samples.

In this study, the results exhibited DNA quantity, the mean of saliva DNA concentrations were (1.23, 2.21, 3.40, 8.24) ng/ μ l to (100,200,300,400) μ l of whole saliva respectively. The mean of buccal swab DNA concentration for same persons was (10.08) ng/ μ l by using Real-Time PCR Table (1). The reason of this difference is due to cell types that found in saliva sample are either epithelial cells [20] or leukocytes and it contains approximately 4.3×10^5 cells per milliliter. Therefore when volume of sample increases, different result is produced [21]. The differences in concentration and purity ratio were depending on the DNA extraction method [20]. Therefore RT-PCR was used to

accurately quantified of DNA, its can start with minimal amounts of nucleic acid. In this study, buccal swab samples were used as reference samples for the genetic analysis. The DNA samples were extracted and quantified, a small amount approximately 1ng of DNA was used for STR procedure, the results are similar in each sample with different volumes [100,200,300,400] μ l as well as buccal swab samples Table (2).

Table (1)
Quantification of human DNA concentration from whole saliva in different volumes and buccal swab by RT-PCR.

No. Sample	DNA concentration (ng\µl) (Volume 100µl)	DNA concentration (ng\µl) (Volume 200µl)	DNA concentration (ng\µl) (Volume 300µl)	DNA concentration (ng\µl) (Volume 400µl)	DNA concentration (ng\µl) Buccal swab
1	4	6.43	8.9	18.7	5.14
2	3.8	7.1	9.2	15.6	15.97
3	0.41	0.06	0.04	2.59	11.18
4	0.03	0.15	2.14	3.01	9.28
5	0.16	0.36	0.62	3.03	13.23
6	0.18	0.9	2.14	8.1	2.7
7	0.08	0.51	0.81	6.7	13.09
Mean of DNA concentration	1.23	2.21	3.40	8.24	10.08

Because of DNA is packaged into pairs of chromosomes, the occurrence of two alleles is produced when the fragment size (allele) at one locus on one chromosome differs from the fragment size (allele) of the same locus on the other chromosome person will have two allele (heterozygous). If the sizes of the detected fragments are the same on both chromosomes, then a person will only have one allele at that locus (homozygous) [22]. STR systems detect DNA at several different locations on the DNA strand at each of these locations (loci); the alleles (A1, A2) of 15 STR loci were not

appeared in most results especially in different volumes samples. Results were appeared (73.3, 83.7, 89.5, 99.04) % respectively in all 15 loci Table (3).

In the present study, minimal amounts (100-200) µl was sufficient for PCR amplification, typing and can be used as source for biomarker profiling and forensic identification, that corresponds to the study of Sweet *et al.*, (1999) [22]. Table 3 demonstrates the percentage of number of STR loci which emerged for each profile in this study.

Table (2)
Alleles of AmpF1STR1 Identifier-TMPCR Amplification Kit loci in saliva samples of 15 STR loci (the results are similar in each sample with different volumes [100, 200, 300, 400] µl as well as buccal swab samples.

STR Locus	Sample (1)		Sample (2)		Sample (3)		Sample (4)		Sample (5)		Sample (6)		Sample (7)	
	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2
D851179	11	14	14	15	14	15	14	14	13	14	14	14	14	15
D21S11	28	30	28	29	28	33.2	29	30	30	32.2	29	30.2	29	32.2
D7S820	10	11	10	10	10	10	8	10	8	10	10	11	10	12
CSF1PO	9	10	12	12	11	12	12	12	10	10	7	11	12	12
D3S1358	15	17	15	18	15	16	15	18	14	16	15	15	16	16
THO1	6	8	6	7	7	9	6	7	6	9	6	9	6	9.3
D13S317	11	13	9	13	9	11	9	10	8	12	8	12	8	10
D16S539	9	11	11	13	8	11	8	13	11	14	10	12	12	13
D2S1338	20	25	20	20	17	14	17	23	22	23	16	17	19	20
D19S433	13	15	13	14	15	15.2	12	15	13	14	14	14	13	15.2
VwA	16	17	15	16	17	17	17	16	16	16	17	17	15	15
Tpox	8	12	9	11	11	11	8	8	8	12	7	8	8	11
D18S51	14	16	14	15	12	15	12	18	14	18	14	17	14	14
D5818	9	12	10	11	9	13	10	10	11	13	9	13	9	10
FGA	24	24	21	22	24	24	22	25	22	23	22	22	23	28

15 STR loci of AmpF1STR1 Identifier-TMPCR Amplification Kit (D851179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, VwA, Tpox, D18S51, D5818, FGA), the alleles1 (A1), alleles 2 (A2).

Table (3)

The Alleles percentage of 15 STR loci that appeared in each profile of different volumes of saliva samples (100, 200, 300, 400) μ l respectively.

STR Locus	Saliva sample (Volume 100 μ l) %	Saliva sample (Volume 200 μ l) %	Saliva sample (Volume 300 μ l) %	Saliva sample (Volume 400 μ l) %
Sample (1)	93.3	100	100	100
Sample (2)	93.3	100	100	100
Sample (3)	66.6	66.6	66.6	93.3
Sample (4)	53.3	73.3	100	100
Sample (5)	66.6	80	86.6	100
Sample (6)	80	80	93.3	100
Sample (7)	60	86.6	80	100
Mean percentage	73.3	83.7	89.5	99.04

Oral fluid sampling is safe for the operator; it has easy and low-cost storage, these characteristics make it possible to monitor several biomarkers in infants, children, elderly and non-collaborative subjects, and in many circumstances in which blood and urine sampling is not available. Another reason that makes saliva interesting for diagnostic purposes is the linkage with traditional biochemical parameters which appear in the circulation in various forms [2]. The above reasons explain why this type of biological sample was selected. The major advantages of

saliva over blood when used for diagnostic purposes include easy access, non-invasive collection, and better patient/subject compliance [23]. In this study, performance characteristics comparison between blood and saliva as biological source were in agreement with Fanyue *et al.*, 2014 [24], can be appeared briefly in the Table (4).

Table (4)

Comparison between blood and saliva as biological source in the performance characteristics (Fanyue *et al.*, 2014).

Performance characteristics	DNA obtain from blood	DNA obtain from saliva
Sample collection	Sometime difficult collection (biopsy vein)	Easy collection (self-administration possible)
Sample storage	Yield decreases at high temperature (without stabilizing agent)	Stable at high temperature
Compliance of study topics	Less compatibility, especially in children; more invasive	More compatibility; less invasive
DNA quality and quantity	High quality and no foreign DNA contamination; high amounts of DNA	Variable quality; may contain foreign DNA or foreign substances; good amounts
PCR based analysis	Good quality if we avoided the effect of the heme, that which can discouraged PCR amplification	Good quality

The concentration of DNA extracted from 200 μ l (saliva, blood) was measured by using Nano-Drop spectrophotometer Table (5). Generally, the mean DNA concentration of blood higher than the saliva (51.164, 10.736) ng/ μ l respectively, These results are in agreement with Hu *et al.*, 2012(25), and Abraham *et al.*, 2012 [26], who reported

that the mean concentration DNA of blood (253.63 μ g per 8.5 ml sample), (26 μ g/ml) respectively, While the mean concentration DNA of saliva (21.09 μ g per 0.5 ml), (12 μ g/ml), respectively.

Table (5)
Concentration of DNA obtained from blood and saliva samples in ng/μL and purity at optical density (OD) 260/280.

No. Sample	Blood sample		Saliva sample	
	Concentration (ng/μl)	Purity(OD) (260\280)	Concentration (ng/μl)	Purity (OD) (260\280)
Mean DNA Concentration (ng/μl)	51.164	1.72	10.736	1.64

The results demonstrate that blood samples yield more DNA than saliva samples, which are mainly due to the peripheral blood typically contains $(4.5 - 11 \times 10^5)$ white blood cells yielding 10-18 μg/ml of genomic DNA [27]. Whereas the mean number of epithelial cells per 1 mL of saliva is about (4.3×10^5) cell, as well as the amount of leukocytes is likely to vary greatly depending on the health status of the donor and the quality of genomic DNA is high without contamination with foreign DNA [21].

Conclusion

The saliva sample is an upcoming area of research for basic and clinical application, it's a biological fluid that offers several opportunities for diagnosis in forensic science. Therefore, this study illustrated the possibility of using saliva samples instead of blood samples in the field of criminal research through access to hereditary profiles from different volumes of saliva sample.

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الخلاصة

يعد جمع وحفظ وتحليل سوائل الجسم جانبا مهما في العلم الجنائي، حيث أصبح الحامض النووي الرايبوزي منقصوص الاوكسجين (الدنا) المعزول من اللعاب بديلا جيدا عن الدم في الدراسات الجينية ويستخدم الآن على نطاق واسع في العديد من التطبيقات. الهدف من هذه الدراسة اولا، هو تحديد مدى ملائمة اللعاب لاستخلاص الحامض النووي و إمكانية استخدامه لتضخيم المؤشرات الجسمية الخاصة بتحديد التتابعات الوراثية الجنائية بالاعتماد على اخذ مسحة فموية باعتبارها المصدر الرئيسي للمقارنة. ثانيا، المقارنة بين الدم واللعاب كمصدر بايولوجي من حيث خصائص الأداء (جمع العينة، حفظ العينة... الخ). تم جمع عينات من المسحة الفموية واللعاب من 7 متطوعين، العينات أخذت بأحجام مختلفة من اللعاب من كل متطوع

(400,300,200,100) ميكرو لتر وبالتالي فإن العدد النهائي للعينات التي استخدمت في هذه الدراسة 28 عينة، كذلك تم جمع عينات من الدم واللعاب من 25 متطوعا كدراسة لمقارنة الخصائص. استخلصت العينات بالاعتماد على طريقة الفينول الكلوروفورم. أظهرت النتائج وجود فرق في تراكيز الحامض النووي المستخلص عند الحصول على نتائج الحامض النووي الكمي باستخدام تقنية Real time - PCR باحجام مختلفة لعينات اللعاب (400، 300، 200، 100) ميكرو لتر (1.23، 2.21، 3.40، 8.24) نانوغرام/ ميكرو لتر على التوالي، كذلك في نتائج تضخم المؤشرات الجسمية الخاصة بتحديد التتابعات الوراثية الجنائية باستخدام جهاز التحليل الوراثي حيث ظهرت نتائج النسبة المئوية لعينات اللعاب باحجام مختلفة (73.3، 83.7، 89.5، 99.04) % على التوالي في 15 موقع. تم تقييم الحامض النووي وفق التركيز والنقاوة وامكانية استخدامه لاغراض التحليلات الوراثية بالنسبة لعينات الدم واللعاب حيث تم قياسها باستخدام جهاز الطيف المرئي (Nano drop) (51.164، 10.736) نانوغرام/ ميكرو لتر (1.72، 1.64) على التوالي. ويستنتج من هذه الدراسة امكانية استخدام اللعاب في مجال البحث الجنائي وامكانية الحصول على الهوية الوراثية باحجام مختلفة من عينات اللعاب، بالاضافة الى امكانية الاستغناء عن اخذ عينات من الدم واستبدالها بعينات اللعاب.

الكلمات المفتاحية: اللعاب، الدم، الحامض النووي، المؤشرات الجسمية الخاصة بتحديد التتابعات الوراثية الجنائية.