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Comparison Between Currently Used Blood Samples And New Saliva Dna Collection Method For Quality Of Genomic Dna And Genotyping

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ABSTRACT. Obtaining blood biospecimens presents logistical and financial challenges. As a result, saliva biospecimen collection is becoming more frequent because of the ease of collection and lower cost. This article describes an assessment of two different methods for collecting samples: whole blood and whole saliva samples used further for DNA extraction and HLA genotyping in immunogenic disease on a group of patients registered at our Molecular Genetics Laboratory Faculty of Medicine "Ovidius" University Constanța. Our data show that only 81% of the requested participants delivered a blood sample, whereas delivered a saliva sample because they refuse the st sampling method. Analysis of purified genomic DN by Nano Photometer and agarose gel el trophoresi revealed that blood and saliva samples sulte in DNA with the best quality. PCR analysi showed t t DNA from 100% of the blood samples d 93% o liva samples could be subsequen y an ifie Our study shows that the response e of self- ection saliva samples had to be cons g for the pati s that have a low response rate blood ampling. The uality of genomic DNA f om saliva sa les was comparable with blood sam es as assessed by ity, concentration, yield and P amplification. We con ude that the use of saliva mples is good alternative to blood samples to obtain nom DNA of high quality and it will con derably ease the p icipant's response rate for enetic udies.

Key blood samples, saliva samples, DNA ollection methods, DNA extraction

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Introduction

Currently, EDTA stabilized whole blood is the most common sample type used for obtaining hi purity DNA. Blood has proven a very consistent ar reliable sou e of genetic material for many ave es of testin and research, but it can also be a time c sumi g, expensive and invasive collection ethod - especially for long term or broad range Scientist are trying to find a comparable source of genetic material, such as saliva, that is more cost effective, more stable and less invasive[1,2]. Large population-based studies involving thousands of participants are needed in the search for genetic determinants underlying common diseases such as cardiovascular diseases, cancer diseases, osteoporosis, and diabetes. Therefore, increasingly epidemiologic studies are trying to supplement survey data with genomic DNA[3]. However, collection of blood samples may not be feasible in large epidemiologic studies where participants are dispersed all over the country or because the method requires venepuncture done by trained staff, making collection of blood samples prohibitively expensive. Furthermore, study subjects may be reluctant to provide blood samples, thereby reducing participation rates. Therefore, less invasive and more cost-efficient procedures for collecting DNA are needed[4]. Several studies have found that exfoliated buccal epithelial cells are promising alternative sources of DNA. Different protocols to obtain genomic DNA have been evaluated. Some studies have found that mouthwash samples yield high amounts of high-quality genomic

DNA [5]. Recently, a Swedish study has tested a new method for self-collection of saliva, Oragene, and has found that Oragene saliva samples from men is of high quality and can be used as an alternative to blood DNA in epidemiologic studies [6]. The purpose of our study was to evaluate the DNA quantity and quality by using different methods of DNA collection and to assess to what extent the collection of DNA material affects the quality and its use in subsequent applications.

Materials and Methods

The subjects included in this study comprise 152 patients with different immunogenetic affections (diabetes mellitus, autoimmune thiroiditis) registered at our Molecular Genetics Laboratory Faculty of Medicine "Ovidius" University Constanța. 28 pa[illegible] refused blood collections (most of them children) nd saliva self collection kit was offered as an alternat[illegible]e. Participants gave written informed [illegible]sent a[illegible] provided samples for the collection [illegible]ethod [illegible]ecified.

*Blood Sample*s: Venous [illegible]lood sam[illegible]es were collected in 2 ml EDTA collecti[illegible]ubes [illegible] were stored at 4°C until D[illegible]A extrac[illegible].

Saliva Sampl[illegible] Whole [illegible]aliva was collected using the [illegible]rage[illegible]™ DNA sel[illegible]ollection kit following the manufac[illegible]r's instruction [7]. Participants w[illegible]e asked to rub t[illegible]ir tongues around the inside o[illegible]their mo[illegible]ths for abou[illegible]15 sec and then deposit a[illegible]roximat[illegible]y 2 ml saliva into the collection cup. When [illegible]equate sa[illegible]ple was collected, the ca[illegible] place[illegible]on the [illegible]al and closed firmly. The [illegible]llecti[illegible] cup is [illegible]i[illegible]ed so that solution from the vial's l[illegible]wer compartment is released and mixes with[illegible]e sa[illegible] hen the cap is securely fastened. [illegible] starts the initial phase of DNA isolation, and stab[illegible]es the saliva sample for long-term storage at room t[illegible]perature or in low-temperature freezers.

DNA *isolation tehnique*. For the first method of sampling genomic DNA was extracted from EDTA-anticoagulated venous blood following the manufacturer's instructions with the QIAmp DNA Blood Mini Kit. Briefly Qiagen QIAamp® DNA Mini Kit, is using spin columns containing a silica-bead membrane where nucleic acids are attracted under high salt concentrations. Furthermore the sample and lysis buffer are added to a sterile tube lysate and is combined with alcohol and placed [illegible]to th[illegible]pin columns. The removal of proteins [illegible] accomplish[illegible] using multiple buffer washes a[illegible] centrifugatio[illegible] steps. Pure DNA is eluted fr[illegible] the n[illegible]brane int[illegible] sterile water or TE buffer [illegible] mmol/L Tr[illegible]HCl [illegible] mmol/L EDTA (pH 8.0[illegible] The DN[illegible] was qu[illegible]ified and stored at −20°C unti[illegible]CR a[illegible]ysis.

DNA was [illegible]racted [illegible]n saliva s[illegible]ples using the Oragene ki[illegible]NA Genote[illegible]as d[illegible]cribed by the manufacture[illegible]Brie[illegible] the Oragen[illegible]iva sample was incubated at 50°C ove[illegible]ight. Five-hundred-microliter sampl[illegible] transferred [illegible]a 1.5 mL Eppendorf tube, 20[illegible]L of Oragene purifier were added, and the sa[illegible]ple was mix[illegible]d by inversion and incubated on ic[illegible] for 10 min. [illegible]he sample was then centrifuged for [illegible] min at 13[illegible]00 rpm at room temperature and the su[illegible]ate[illegible] was transferred to a new tube. Five [illegible]ndred microliters of 95% ethanol were added; the [illegible]mp[illegible]as mixed by inversion at least five times and incubated at 10 min at room temperature. The sample was then centrifuged for 1 min at 13,000 rpm at room temperature, the supernatant was discarded, and the DNA was dissolved in 100 µL TE buffer and quantified. The DNA samples were stored at −20°C until PCR analysis.

DNA *quantification and quality determinations*. We first compared the quantity and purity of isolated genomic DNA from both the blood and saliva samples. As shown in Table 1, the purity of genomic DNA extracted from the saliva samples is not significantly different than that from the blood samples. However, the DNA yield from saliva samples is lower when compared to the blood samples.

The concentration of 3 µL DNA sample was determined using NanoPhotometer spectrophotometer (Figure no.1). Absorbance of ultraviolet light at wavelengths of 230, 260, and 280 nanometers was used to calculate the OD260/OD280 and OD260/OD230 ratios to compare the ratio of nucleic acid concentration in the sample (OD260) to that of protein and organics (OD280), and salt and alcohol (OD230) contaminants. A ratio of 1.5–2 is generally

Table I - The average parameters of DNA extracted from blood and saliva samples and their respective standard deviations. The mean values of the 260/280 and 260/230 nm ratios were 1.62/1.37 and 1.78/1.12 for genomic DNA purified from blood and saliva

	A260/ A280 +/- SD	Concentration (ng/ ul) +/- SD	Yield (µg) +/- SD	A260/ A230 +/- SD	PCR Amplification
	Acceptable range: 1.5-2.0	***Acceptable range: > 50***	***Acceptable range: >2***	***Acceptable range: >1.2***	
Blood	1.62 +/- 0.09	57 +/- 30.67	5.93 +/- 2.13	1,37 +/-0.09	100%
Saliva	1.78 +/- 0.08	47 +/- 43.81	4.78+/- 3.26	1.12+/- 0	93%

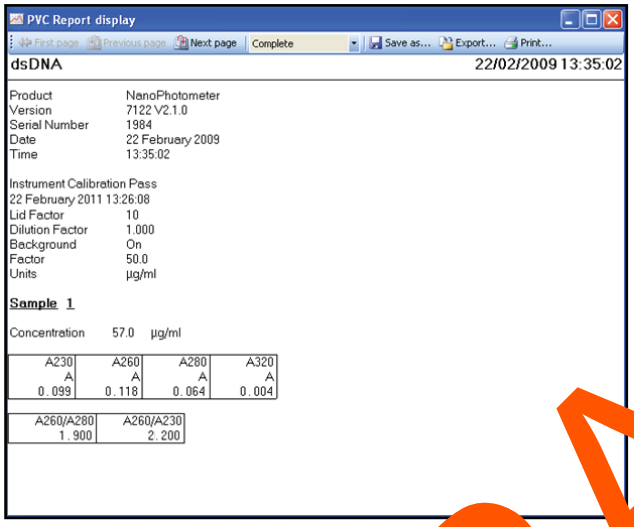

Figure 1 - Example of a blood sam[illegible] anal[illegible]d by NanoPhotometer showing a concen[illegible]tion of [illegible]A of 57 ng/µL and OD260/OD280 ratio of [illegible]0 (in th[illegible] range 1.[illegible]2.0)

preferred for the OD2[illegible]/OD280 ra[illegible] (indicating limited protein and [illegible]orga[illegible]c contamina[illegible]on), and values higher than 1.2 are p[illegible]rred for the OD260/ OD230 ratio [illegible]dicating limite[illegible] salt and alcohol contaminati[illegible]). Moreover DNA is[illegible]ated from blood samples [illegible]d DNA [illegible]olated from saliva was loaded on a 1% ag[illegible]os[illegible]gel (8µL [illegible]NA solution/well) and vis[illegible]d by [illegible]idium b[illegible]mide staining.

[illegible]CR *Am[illegible]fic[illegible]ion*. The isolated DNA was amplifi[illegible] by ESSC Thermal Cycler PCR (using [illegible]he [illegible]Stocki[illegible]cording to the manufacturer) to c[illegible]rm its utility in HLA typing with Sequence-Spe[illegible]c Oligonucleotide (SSO) or Sequence-Specifi[illegible] Primers (SSP). The number and percentage of successful hybridization products obtained by PCR amplifications were recorded for each set of samples and were traced by colorimetric-detection systems (streptavidin-biotin) for SSO method and by gel electrophoresis f[illegible]SSP me[illegible]od as p[illegible]ted elsewhere [8].

Results

All samples were [illegible]alyzed for concentration (n[illegible]µL), purity ([illegible]260/A280 ratios) and yield (µg) on a [illegible]anoPhotomet[illegible] as well as for integrity on a 1% aga[illegible]e gel.

[illegible]l[illegible]e[illegible]mated amount of total DNA extracted [illegible]m 200 mL blood samples varied between 3.8 and [illegible]00 [illegible]with a mean of 5.93 µg, and from 0.5 mL Oragene saliva samples between 1.52 and 8.04µg with a mean of 4.78 µg. DNA quality can be affected by collection method (primarily integrity and protein contamination) and by isolation method (integrity and protein, organic, salt, and alcohol contamination). As shown in Table 1, mean of median OD260/OD280 ratios for method fell within the criterion range (1.5–2.0), indicating acceptably low protein and organic contamination of the DNA products. The whole-saliva method had somewhat greater overall salt and alcohol contamination (range, 0.66–1.53; median, 1.12)

In PCR amplification all 152 (100%) blood samples and only 17 (93%) saliva samples were amplified. The two saliva samples that could not be amplified had a low concentration DNA, and we had to repeat the extraction with elution in 35 µL TE. Second extraction produced a high enough DNA concentration for subsequent amplification. The DNA was further examined by agarose gel electrophoresis (Figure no.2). For blood and saliva samples, a visible band of high molecular weight DNA and a smear over a broad size range was observed.

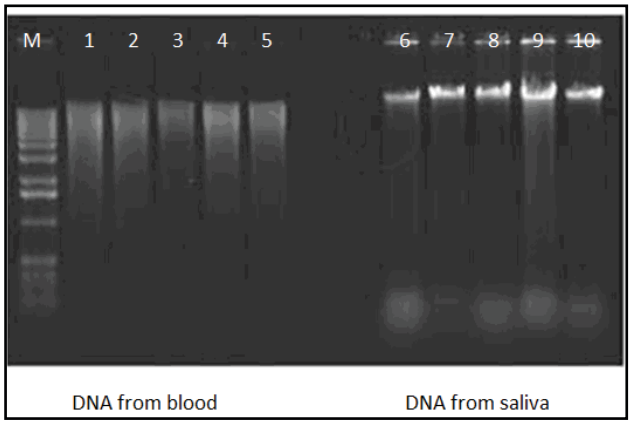

Figure 2 - Electrophoretic analysis of genomic DNA from blood and saliva. DNA (8µL) was loaded on a 1% agarose gel and visualized by ethidium bromide staining. The gel shows in the first lane a dimension marker and in next ten lanes samples of DNA from blood (1-5) and, respectively, from saliva (6-10)

Discussion

Simple, self-administrated sample collection method, saliva increase participation rate significantly in particular among children or patients that are complying about blood collection method. The mean DNA yield from 200 µL blood was 22 µg, whereas 0.5 mL Oragene saliva sample resulted in a mean DNA yield of 4.78 µg, which is lower than recently published data [9]. However in these studies, DNA was purified from 2 mL Oragene saliva sample. Because the average amount of saliva sample in our study was ~ 4mL (including buffer), we will be able to purify significantly larger amounts of DNA if necessary.

We did not examine the amount of bacterial DNA present in our samples. However, it is well known that buccal and saliva samples are contaminated with bacterial DNA. Bacterial contamination primarily depends on the way the samples are kept after collection. However, the Oragene sample kit contains an antibacterial agent, which prevents the growth of bacteria between the time of collection and the time of DNA purification. Previous studies have shown that swabs/cytobrushes contain only 11% human DNA, whereas mouthwash samples contain 34% to 49% of human DNA. In contrast, saliva samples contained an average human DNA yield of 68% [9]. However, there is concern, of point source microbial contamination inherent in the human saliva and how it may interfere with array genotyping rates, even though the human DNA could be specifically quantified.

The quality of genomic DNA was examined by spectrophotometer, whereas the peak of UV light absorption for DNA is 260 nm, the peak of UV light for proteins is 280 nm, whereas absorption at 230 nm reflects impurities of, for example, carbohydrates, peptides, phenols, buffer salts and other aromatic compounds. The 260/280 nm ratios from DNA from blood were on average 1.63, whereas the average ratios from DNA from saliva samples were 1.79. This suggests that these samples are contaminated with proteins which can overestimate the amount of DNA in these samples.

The results show that all blood samples could be amplified, and whereas 93% of the saliva samples could be amplified. The genotyping result is lower than the previously published result of 96% [9] and the difference could be due to the use of different DNA extraction methods.

After repeating extraction for two samples, the entire DNA from the blood and from saliva samples could moreover be genotyped. These results indicate that saliva samples—besides genotyping analysis can be used in mutational screening of disease-causing genes.

Yielding good DNA quality, we suggest that saliva samples are a good alternative to blood samples especially in epidemiologic studies.

References

1. FREEMAN B, POWELL J, BALL D, HILL L, CRAIG I, PLOMIN R. DNA by mail: an inexpensive and noninvasive method for collecting DNA samples from widely dispersed populations. Behav Genet. 1997;27:251–257.
2. KING IB, SATIA-ABOUTA J, THORNQUIST MD, BIGLER J, PATTERSON RE, KRISTAL AR, SHATTUCK AL, POTTER JD, WHITE E. Buccal cell DNA yield, quality, and collection costs: Comparison of methods for large-scale studies. Cancer Epidemiol Biomarkers Prev. 2002;11:1130–1133.
3. HANSEN TV, SIMONSEN MK, NIELSEN FC, HUNDRUP YA (2007) Collection of blood, saliva, and buccal cell samples in a pilot study on the Danish nurse cohort: comparison of the response rate and quality of genomic DNA. Cancer Epidemiol Biomarkers Prev 16: 2072–2076
4. LE MARCHAND L, LUM-JONES A, SALTZMAN B, VISAYA V, NOMURA AI KOLONEL LN. Feasibility of collecting buccal cell DNA by mail in a cohort study. Cancer Epidemiol Biomarkers Prev. 2001;10:701–703.
5. FEIGELSON HS, RODRIGUEZ C, ROBERTSON AS, JACOBS EJ, CALLE EE, REID YA, THUN MJ. Determinants of DNA yield and quality from buccal cell samples collected with mouthwash. Cancer Epidemiol Biomarkers Prev. 2001;10:1005–1008.
6. RYLANDER-RUDQVIST T, HAKANSSON N, TYBRING G, WOLK A. Quality and quantity of saliva DNA obtained from the self-administrated oragene method—a pilot study on the cohort of Swedish men. Cancer Epidemiol Biomarkers Prev 2006;15:1742–5.
7. DNA GENOTEK INC. ORAGENE™ DNA self-collection kit. DNA Genotek, Inc.; Ottawa: 2004b.
8. DURBALĂ Assessment of the risk for type 1 diabetes mellitus conferred by HLA class II genes. Annals of the Romanian Society for Cell Biology. 2009; 14(2):123-9.

BIRNBOIM HC. DNA yield with Oragene™. DNA Genotek, Inc.; Ottawa: 2004b.