

Decizie de indexare a faptei de plagiat la poziția 00160 / 17.04.2015 și pentru admitere la publicare în volum tipărit

care se bazează pe:

A. Nota de constatare și confirmare a indiciilor de plagiat prin fișa suspiciunii
 inclusă în decizie.

Fișa suspiciunii de plagiat / Sheet of plagiarism's suspicion			
Opera suspicionată (OS)		Opera autentică (OA)	
Suspicious work		Authentic work	
OS	AFTENIE, Loredana Mariana, FRANCIUC , Irina, MARTINESCU, Alina, and HONCEA, Adina. Comparison Between Currently Used Blood Samples And New Saliva Dna Collection Method For Quality Of Genomic DNA And Genotyping. <i>ARS Medica Tomitana</i> . 2012 Dec 1. 18 (1). pp.19-23.		
OA	HANSEN, TV, SIMONSEN, MK, NIELSEN, FC, HUNDRUP, YA. 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. <i>Cancer Epidemiology and Prevention Biomarkers</i> . 2007 Oct 1; 16 (10). pp.2072-2076.		
Incidența minimă a suspiciunii / Minimum incidence of suspicion			
P01:	p.19:12d – p.20:10s	P01.01:	p.2072:02s – p.2072:08s
		P01.02:	p.2072:11s – p.2072:06d
		P01.03:	p.2072:08d – p.2072:00d
P02:	p.20:13d – p.20:30d	P02:	p.2073:08d – p.2073:27d
Fișa întocmită pentru includerea suspiciunii în Indexul Operelor Plagiate în România de la Sheet drawn up for including the suspicion in the Index of Plagiarized Works in Romania at www.plagiate.ro			

Notă: Prin „p.72:00” se înțelege paragraful care se termină la finele pag.72. Notăția „p.00:00” semnifică până la ultima pagină a capitolului curent, în întregime de la punctul inițial al preluării.

Note: By „p.72:00” one understands the text ending with the end of the page 72. By „p.00:00” one understands the taking over from the initial point till the last page of the current chapter, entirely.

B. Fișa de argumentare a calificării de plagiat alăturată, fișă care la rândul său este parte a deciziei.

Echipa Indexului Operelor Plagiate în România

Fișa de argumentare a calificării

Nr. crt.	Descrierea situației care este încadrată drept plagiat	Se confirmă
1.	Preluarea identică a unor pasaje (piese de creație de tip text) dintr-o operă autentică publicată, fără precizarea întinderii și menționarea provenienței și însușirea acestora într-o lucrare ulterioară celei autentice.	✓
2.	Preluarea a unor pasaje (piese de creație de tip text) dintr-o operă autentică publicată, care sunt rezumate ale unor opere anterioare operei autentice, fără precizarea întinderii și menționarea provenienței și însușirea acestora într-o lucrare ulterioară celei autentice.	
3.	Preluarea identică a unor figuri (piese de creație de tip grafic) dintr-o operă autentică publicată, fără menționarea provenienței și însușirea acestora într-o lucrare ulterioară celei autentice.	
4.	Preluarea identică a unor tabele (piese de creație de tip structură de informație) dintr-o operă autentică publicată, fără menționarea provenienței și însușirea acestora într-o lucrare ulterioară celei autentice.	
5.	Republicarea unei opere anterioare publicate, prin includerea unui nou autor sau de noi autori fără contribuție explicită în lista de autori	
6.	Republicarea unei opere anterioare publicate, prin excluderea unui autor sau a unor autori din lista inițială de autori.	
7.	Preluarea identică de pasaje (piese de creație) dintr-o operă autentică publicată, fără precizarea întinderii și menționarea provenienței, fără nici o intervenție personală care să justifice exemplificarea sau critica prin aportul creator al autorului care preia și însușirea acestora într-o lucrare ulterioară celei autentice.	✓
8.	Preluarea identică de figuri sau reprezentări grafice (piese de creație de tip grafic) dintr-o operă autentică publicată, fără menționarea provenienței, fără nici o intervenție care să justifice exemplificarea sau critica prin aportul creator al autorului care preia și însușirea acestora într-o lucrare ulterioară celei autentice.	
9.	Preluarea identică de tabele (piese de creație de tip structură de informație) dintr-o operă autentică publicată, fără menționarea provenienței, fără nici o intervenție care să justifice exemplificarea sau critica prin aportul creator al autorului care preia și însușirea acestora într-o lucrare ulterioară celei autentice.	
10.	Preluarea identică a unor fragmente de demonstrație sau de deducere a unor relații matematice care nu se justifică în regăsirea unei relații matematice finale necesare aplicării efective dintr-o operă autentică publicată, fără menționarea provenienței, fără nici o intervenție care să justifice exemplificarea sau critica prin aportul creator al autorului care preia și însușirea acestora într-o lucrare ulterioară celei autentice.	
11.	Preluarea identică a textului (piese de creație de tip text) unei lucrări publicate anterior sau simultan, cu același titlu sau cu titlu similar, de un același autor / un același grup de autori în publicații sau edituri diferite.	
12.	Preluarea identică de pasaje (piese de creație de tip text) ale unui cuvânt înainte sau ale unei prefețe care se referă la două opere, diferite, publicate în două momente diferite de timp.	

Alte argumente particulare: a) Deși nu citează Tabelul 1 scrierea plagiată îi schimbă sursa față de scrierea autentică. b) Piesa P01 cuprinde un citat menționat în scrierea autentică pe care scrierea plagiată nu-l mai identifică.

Notă:

a) Prin „proveniență” se înțelege informația din care se pot identifica cel puțin numele autorului / autorilor, titlul operei, anul apariției.

b) Plagiul este definit prin textul legii¹.

„...plagiul – expunerea într-o operă scrisă sau o comunicare orală, inclusiv în format electronic, a unor texte, idei, demonstrații, date, ipoteze, teorii, rezultate ori metode științifice extrase din opere scrise, inclusiv în format electronic, ale altor autori, fără a menționa acest lucru și fără a face trimitere la operele originale...”.

Tehnic, plagiul are la bază conceptul de **piesă de creație** care²:

„...este un element de comunicare prezentat în formă scrisă, ca text, imagine sau combinat, care posedă un subiect, o organizare sau o construcție logică și de argumentare care presupune niște premise, un raționament și o concluzie. Piesa de creație presupune în mod necesar o formă de exprimare specifică unei persoane. Piesa de creație se poate asocia cu întreaga operă autentică sau cu o parte a acesteia...”

cu care se poate face identificarea operei plagiate sau suspectate de plagiat³:

„...O operă de creație se găsește în poziția de operă plagiată sau operă suspectată de plagiat în raport cu o altă operă considerată autentică dacă:

- Cele două opere tratează același subiect sau subiecte înrudite.
- Opera autentică a fost făcută publică anterior operei suspectate.
- Cele două opere conțin piese de creație identificabile comune care posedă, fiecare în parte, un subiect și o formă de prezentare bine definită.
- Pentru piesele de creație comune, adică prezente în opera autentică și în opera suspectată, nu există o menționare explicită a provenienței. Menționarea provenienței se face printr-o citare care permite identificarea piesei de creație preluate din opera autentică.
- Simpla menționare a titlului unei opere autentice într-un capitol de bibliografie sau similar acestuia fără delimitarea întinderii preluării nu este de natură să evite punerea în discuție a suspiciunii de plagiat.
- Piesele de creație preluate din opera autentică se utilizează la construcții realizate prin juxtapunere fără ca acestea să fie tratate de autorul operei suspectate prin poziția sa explicită.
- În opera suspectată se identifică un fir sau mai multe fire logice de argumentare și tratate care leagă aceleași premise cu aceleași concluzii ca în opera autentică...”

¹ Legea nr. 206/2004 privind buna conduită în cercetarea științifică, dezvoltarea tehnologică și inovare, publicată în Monitorul Oficial al României, Partea I, nr. 505 din 4 iunie 2004

² ISOC, D. Ghid de acțiune împotriva plagiatului: bună-conduită, prevenire, combatere. Cluj-Napoca: Ecou Transilvan, 2012.

³ ISOC, D. Prevenitor de plagiat. Cluj-Napoca: Ecou Transilvan, 2014.



Retraction of:

Aftenie Loredana Mariana¹, Franciuc Irina¹, Martinescu Alina², Honcea Adina²

Comparison between currently used blood samples and new saliva DNA Collection Method for quality of Genomic DNA and Genotyping

ARS Medica Tomitana - 2012; 1(68); Pages 19-23. Feb 2012

(DOI 10.2478/v10307-012-0003-0)

The article is emphasizing the original results of the authors within the Molecular Genetic Laboratory, using specific extraction methods and common international protocols. Due to these procedures, similarities between syntax and expressions may appear with literature. Thus, in order to avoid any confusion, the authors have requested that their article be withdrawn.

DOI: 10.1515/arasm-2017-0097

Aftenie Loredana Mariana¹, Franciuc Irina¹, Martinescu Alina², Honcea Adina²

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

¹Cellular and Molecular Biology Department, Faculty of Medicine, “Ovidius” University Constanța

²Medical Genetics Department, Faculty of Medicine, “Ovidius” University Constanța

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 19% delivered a saliva sample because they refuse the first sampling method. Analysis of purified genomic DNA by Nano Photometer and agarose gel electrophoresis revealed that blood and saliva samples resulted in DNA with the best quality. PCR analysis showed that DNA from 100% of the blood samples and 93% of the saliva samples could be subsequently amplified. Our study shows that the response rate of self-collection saliva samples had to be considering for the patients that have a low response rate of blood sampling. The quality of genomic DNA from saliva samples was comparable with blood samples as assessed by purity, concentration, yield and PCR amplification. We conclude that the use of saliva samples is a good alternative to blood samples to obtain genomic DNA of high quality and it will considerably increase the participant’s response rate for genetic studies.

Key words: blood samples, saliva samples, DNA collection methods, DNA extraction

Aftenie Loredana Mariana MD

Faculty of Medicine, “Ovidius” University, Constanta
Aleea Universității nr.1, 900437 Constanța, România
Tel: +40-723-016-601
E-mail: dana2003_lo@yahoo.ro

Introduction

Currently, EDTA-stabilized whole blood is the most common sample type used for obtaining high purity DNA. Blood has proven a very consistent and reliable source of genetic material for many avenues of testing and research, but it can also be a time consuming, expensive and invasive collection method - especially for long term or broad range studies. 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 thyroiditis) registered at our Molecular Genetics Laboratory Faculty of Medicine “Ovidius” University Constanța. 28 patients refused blood collections (most of them children) and saliva self collection kit was offered as an alternative. Participants gave written informed consent and provided samples for the collection method specified.

Blood Samples: Venous blood samples were collected in 2 ml EDTA collection tubes and samples were stored at 4°C until DNA extraction.

Saliva Samples: Whole saliva was collected using the Oragene™ DNA self-collection kit following the manufacturer’s instruction [7]. Participants were asked to rub their tongues around the inside of their mouths for about 15 sec and then deposit approximately 2 ml saliva into the collection cup. When an adequate sample was collected, the cap was placed on the vial and closed firmly. The collection cup is designed so that solution from the vial’s lower compartment is released and mixes with the saliva when the cap is securely fastened. This starts the initial phase of DNA isolation, and stabilizes the saliva sample for long-term storage at room temperature or in low-temperature freezers.

DNA isolation technique. 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 into the spin columns. The removal of proteins is accomplished using multiple buffer washes and centrifugation steps. Pure DNA is eluted from the membrane into sterile water or TE buffer [10 mmol/L Tris-HCl, 0.1 mmol/L EDTA (pH 8.0)]. The DNA was quantified and stored at –20°C until PCR analysis.

P02 DNA was extracted from saliva samples using the Oragene kit (DNA Genotek) as described by the manufacturer. Briefly, the Oragene saliva sample was incubated at 50°C overnight. Five-hundred-microliter sample was transferred to a 1.5 mL Eppendorf tube, 20 µL of Oragene purifier were added, and the sample was mixed by inversion and incubated on ice for 10 min. The sample was then centrifuged for 3 min at 13,000 rpm at room temperature and the supernatant was transferred to a new tube. Five hundred microliters of 95% ethanol were added; the sample was 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 1 - 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+/- 0.08	93%

PVC Report display				
dsDNA 22/02/2009 13:35:02				
Product	NanoPhotometer			
Version	7122 V2.1.0			
Serial Number	1984			
Date	22 February 2009			
Time	13:35:02			
Instrument Calibration Pass				
22 February 2011 13:26:08				
Lid Factor	10			
Dilution Factor	1.000			
Background	On			
Factor	50.0			
Units	µg/ml			
Sample 1				
Concentration	57.0 µg/ml			
A230	A260	A280	A320	
0.099	0.118	0.064	0.004	
A260/A280		A260/A230		
1.900		2.200		

Figure 1 - Example of a blood sample analyzed by NanoPhotometer showing a concentration of DNA of 57 ng/µL and OD260/OD280 ratio of 1.90 (in the acceptable range 1.5-2.0)

preferred for the OD260/OD280 ratio (indicating limited protein and organic contamination), and values higher than 1.2 are preferred for the OD260/OD230 ratio (indicating limited salt and alcohol contamination). Moreover DNA isolated from blood samples and DNA isolated from saliva was loaded on a 1% agarose gel (8µL DNA solution/well) and visualized by ethidium bromide staining.

PCR Amplification. The isolated DNA was amplified by ESSO Thermal Cycler PCR (using the protocols according to the manufacturer) to confirm its utility in HLA typing with Sequence-Specific Oligonucleotide (SSO) or Sequence-Specific 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 for SSP method as presented elsewhere [8].

Results

All samples were analyzed for concentration (ng/µL), purity (A260/A280 ratios) and yield (µg) on a NanoPhotometer as well as for integrity on a 1% agarose gel.

The estimated amount of total DNA extracted from 200 mL blood samples varied between 3.8 and 8.06 µg 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.

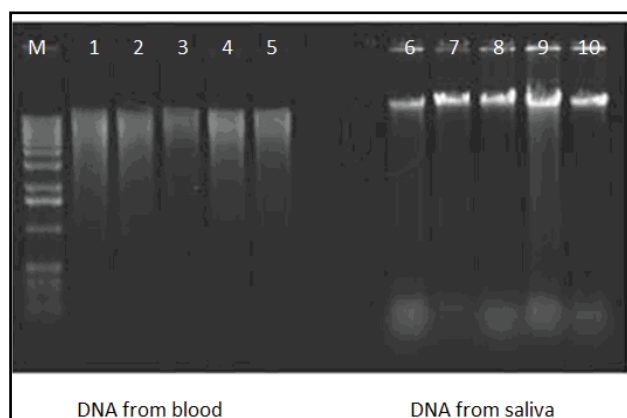


Figure 2 - Electrophoretic analysis of genomic DNA from blood and saliva. DNA (8 μ L) was loaded on a 1% agarose gel and visualized by ethidium bromide staining. The gel shows in the first lane a dimension marker and in next ten lanes samples of DNA from blood (1-5) and, respectively, from saliva (6-10)

Discussion

Simple, self-administrated sample collection method, saliva increase participation rates significantly in particular among children or patients that are complying about blood collection method. The mean DNA yield from 200 mL blood was 5.93 μ g, whereas 0.5 mL Oragene saliva sample resulted in a mean DNA yield of 4.78 μ g, 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 recently 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.