Saliva as a Source of Genomic DNA for Genetic Studies: Review of Current Methods and Applications

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Abstract

DNA 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. This review addresses advantages of saliva DNA for large population-based studies and points out caveats for use in certain techniques. We compiled data comparing saliva-derived genomic DNA to blood-derived DNA with regards to quality, quantity and convenience. Special attention is given to the usefulness of saliva-derived DNA for PCR-based methods and genome-wide analyses.

Key words: saliva, blood, genomic DNA, comparison, genotyping, genome-wide analysis

Introduction

Genetic studies often require the recruitment of large numbers of subjects with certain physical or physiological characteristics and ascertainment of DNA samples. In the past, most blood samples were obtained by venipuncture requiring trained staff. In our experience and in the experience of others, participation rates can be low when phlebotomy is involved since some study subjects may be reluctant to have their blood drawn. Reasons for non-participation if blood drawing is involved include fear of needle stick in healthy individuals, reluctance of going through yet another procedure in subjects with health problems, veins that are difficult to access or hesitation of recruiters and caretakers to draw blood from elderly and feeble individuals or from young children. When there is a choice, most requested participants prefer saliva samples, buccal cell samples via mouth swabs or buccal cell samples on FTA cards [1].

Until recently, blood or tissues were the main sources for isolating sufficient amounts of genomic DNA for genetic studies. Using buccal swaps, preserved dried blood spots, hair, urine, fecal or amniotic samples for DNA isolation often requires whole genome amplification steps to obtain the desired amounts of genomic DNA fragments. Buccal cells have been collected for genetic studies using cytology brushes, mouthwash or treated cards [2]. Cytobrushes provided the best DNA in terms of quality and quantity. While these methods as well as cheek scraping with tongue depressors [3] work well for PCR based experiments, the DNA quality from buccal cells is poor and not suitable for DNA genotyping [1]. A significant disadvantage is the need for whole genome amplification for most genotyping projects [4,5]. In addition, buccal cell samples are thought not to perform accurately for CNV detection because of the abundance of bacterial DNA [6].

Investigators for larger genomic or epidemiologic studies have long been struggling for easy-to-use and preferably non-invasive sample collection methods that yield the desired amounts and quality of genomic DNA [7]. The purity, integrity, and concentration of isolated DNA fragments directly affect the quality of results. A preferred sample source for DNA should fulfill a number of requirements: it should be noninvasive, rapidly and easily obtained, it should maintain DNA integrity during shipment and storage at ambient temperatures (even in the tropics) and it should provide sufficient amounts of high quality DNA for follow-up experiments [8]. There are several commercially available saliva collection kits on the market, which presumably produce comparable results. When choosing a saliva collection system it is important to pay attention to the ease of use of the collection tubes and the recommended storage conditions for samples before DNA extraction. The latter is especially important when samples are to be collected in tropical regions of the world.

Here we discuss the pros and cons of saliva DNA compared to genomic DNA from blood and their suitability for various genetic methods that are commonly used.

Sample storage and shipping

Sample collection is a major cost and time factor, especially when large numbers of samples have to be collected. Storage requirements of acquired samples become especially important for field studies and more so when samples are to be collected in regions of the world where infrastructure for cooling or freezing is lacking and specimens need to be shipped across continents.

Blood samples collected in Vacutainer tubes (e.g., BD Vacutainer® Blood Collection Tube with either Heparin or ACD; BD Biosciences, San Jose, CA) should be refrigerated and DNA should be extracted within weeks of collection for best results. In our experience, the yield of full-length DNA quickly decreases by up to 80% when blood samples are stored for several weeks and shipped at ambient temperature from tropical countries (i.e. 30-33˚C). Blood can be stabilized with reagents such as the DNAgard® Blood stabilization reagent (Biomatrica, San Diego, CA) during shipping and processing for up to 14 weeks at room temperature. However, the use of DNAgard® Blood requires additional handling steps to dilute the blood 4:1 (blood:stabilizer) before storage. Saliva samples that already contain appropriate preservatives on the other hand are stable even at higher ambient temperatures according to manufacturers' assurances (e.g., DNAGenotek

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INC. Kanata, Ontario, Canada; Biomatrica, San Diego, CA, USA; Courtagen Life Sciences Inc. Woburn, MA, USA, etc.).

Several studies attested high DNA quality of saliva samples after 30 days storage at 37°C [8], up to 6 months at room temperature and additional 2 weeks at 30°C [9] or 8 months at room temperature [10]. One company, DNA Genotek (Kanata, Ontario, Canada) claims that high molecular weight DNA is maintained even after 5 years of storage at room temperature, 6 months at 37°C or 3 months at 50°C (http://www.dnagenotek.com/US/pdf/PD-WP-005.pdf)

Both, blood and saliva samples for genetic studies may be shipped as exempt human specimen if not intended for diagnostic purposes and there is only minimal likelihood that pathogens are present. Shipments have to be packed in a way compliant with regulations of the International Air Transport Association (IATA), the International Civil Aviation Organization (ICAO) and the Department of Transportation (DOT) or their foreign equivalents. As with all kinds of liquid samples, tubes must be tightly closed and packaged to avoid breakage. Sample preservative leaking from a tube may not only contaminate other samples but the preservative from saliva samples may erase labeling of other tubes and render samples useless.

Compliance of study subjects

Here we compare the most common methods for obtaining blood-derived and saliva-derived genomic DNA for genetic research. Obtaining blood from patients is most often performed using 10 ml BD Vacutainer® Tubes (BD Biosciences, San Jose, CA) and DNA is isolated with commercially available solutions such as the Gentra Puregene Blood Kit (Qiagen, Valencia, CA), DNA Isolation Kit for Mammalian Blood (Roche, Nutley, NJ) or NucleoSpin Blood XL (Clontech, Mountain View, CA). Saliva samples in our laboratory are usually obtained with Oragene•DNA collection kits (DNAGenotek, Kanata, Ontario, Canada). Costs for DNA isolation (supplies and labor) from commercially available blood collection kits and commercially available saliva kits are similar. Less expensive DNA extraction methods for blood-derived DNA include high-salt extraction methods [8] or traditional phenol-chloroform isoamyl alcohol extraction [11]. Premixed reagents are commercially available (UltraPure™ Phenol:Chloroform:Isoamyl Alcohol (25:24:1, v/v), Invitrogene, Grand Island, NY).

Blood samples for research may be readily obtained in clinical settings when phlebotomy is required for medical reasons. In field studies, blood drawing becomes more challenging as specialized personnel must be available and research subjects, especially unaffected control individuals, may be hesitant to participate. Higher compensation of subjects may be needed to encourage study participation. In contrast, saliva kits can be self-administered by most subjects with the appropriate instructions from the recruiter. Saliva collection may also reduce stress and anxiety in study participants, especially in children and the elderly. Using saliva instead of blood may reduce the exposure of laboratory personnel to biohazards and pathogens. Epidemiologic or case-control studies often require large numbers of samples. For such studies it is not only important to obtain sufficient amounts of high quality DNA but also to ascertain samples from subjects in the most convenient and economical way. Therefore, many large epidemiological, association or population-based studies prefer saliva samples over blood sample collection whenever analysis methods permit [10,12-16]. There are some circumstances when saliva (or buccal cell) DNA is the more appropriate source for germline DNA, for example, in patients with hematologic malignancies [17] or in monozygotic twins that were exposed to fused circulations (feto-fetal transfusion syndrome) [18].

DNA quality and quantity

Peripheral blood typically contains 4.5 to 11 × 10^5 white blood cells yielding 10-18 µg/ml of genomic DNA [19]. The quality of genomic DNA is high without contamination with foreign DNA. The amount of RNA contamination in genomic DNA depends on the DNA extraction method and the skillfulness of the experimenter. Saliva contains approximately 4.3 × 10^5 cells per milliliter [20]. The cell types found in saliva are thought to be either epithelial cells [20] or leukocytes [21]. The amount of leukocytes is likely to vary greatly depending on the health status of the donor. Total DNA derived from saliva samples can be quite variable. Published DNA yields from blood and saliva are summarized in *Table 1*. Note that DNA yields reported in these publications refer either to mean sample volumes, to standardized volumes of 1 ml, or may refer to the total assumed sample volume without stating whether there was any variability in the sample volumes.

However, the overall amount of DNA from blood is usually higher than the total amount of DNA from saliva as usually 8-10 ml of blood are drawn in a Vacutainer tube, while the saliva kits are usually limited to 2-4 ml per tube.

The quality of DNA from blood samples is rarely impacted by foreign DNA while DNA contamination is commonly found in saliva DNA. Previous studies showed that only 50% of the DNA from saliva is of human origin as determined by hybridization to the D17Z1 probe [22], or 68% as determined by real time PCR assessment for the human prothrombin gene [23]. Other studies assessed the mean amplifiable human DNA percentage in saliva samples to 37.3% [24], 40.66% [8] or 77% [15] of total DNA. In contrast, the mean amplifiable human DNA percentage in blood samples is 87.6% [24].

The average bacterial numbers in saliva were estimated to 1.7 × 10^7/ml [20]. Bacteria, fungi and potentially food remnants in the oral cavity contribute to the overall amount of DNA from saliva samples and may affect experimental designs. These contaminants can be reduced by rinsing the mouth thoroughly prior to saliva donation. Recommendations are to thoroughly rinse with water 5 to 30 minutes before saliva donation and abstain from eating and drinking after that. A recent comparison of saliva-derived DNA found that adhering to the manufacturer's volume specification is important [14]. Genotyping call rates were reduced when the Oragene saliva collection tubes were filled with only 0.5 ml saliva instead of the recommended 2 ml and significantly diminished when samples were excessively turbid.

Storage conditions are important considerations for obtaining high quality DNA in field studies. While blood samples are usually best preserved at 4°C for several weeks or in a freezer, preservatives of commercially available saliva samples maintain DNA quality over a surprisingly broad range of storage conditions. Several studies attested high DNA quality of saliva samples after storage of 30 days at

Table 1. *DNA yield from saliva and blood reported by various studies.*

Author	Saliva			Blood		
	DNA yield (µg) ± SD	Range of DNA yield (µg)	% human DNA	DNA yield (µg) ± SD	Range of DNA yield (µg)	% human DNA
Looi et al.2012 [39]	N/A	15.6 per ml sample (µg/ml)	-	N/A	14.8 per ml sample (µg/ml)	-
Abraham et al. 2012 [13]	12 per ml sample (µg/ml)	0.1-26 per ml sample (µg/ml)	-	26 per ml sample (µg/ml)	6-73 per ml sample (µg/ml)	-
Koni et al. 2012 [12]	20.95 ± 2.35 per ml sample (µg/ml)		-	-	-	-
Ng et al. 2006 [9]	17.8 (µg/ml)	4.25-42.6	-	-	-	-
Nunes et al. 2012 [10]	36.6 ± 23.8 per ml sample (µg/ml)	2-160 per ml sample (µg/ml)	-	-	-	-
Rylander-Rudqvist et al. 2006 [23]	40.3 ± 36.5 Per total yield from 1.5-2.5 ml saliva)	1.2-169.7 Per total yield from 1.5-2.5 ml saliva)	68% ± 20% Range 11-100%	-	-	-
Viltrop et al. 2010 [37]	11.9 µg per sample	0.9-36 µg per sample	-	113 µg per sample	-	-
Philibert et al. 2008 [19]	23 ± 18.5 per ml sample (µg/ml)	-	-	18.5 ± 14.3 per ml sample (µg/ml)	-	-
Quinque et al. 2006 [8]	11.4 µg per ml sample (µg/ml)	2.45 – 21.1 per ml sample (µg/ml)	40.66% Range 10-100%	-	-	-
Nishita et al.2009 [15]	82.8 µg per sample (average 4.5 ml sample)	0.9 -549.3 µg	77% ±76.1%	-	-	-
Hu et al. 2012 [24]	21.09 ± 3.64 µg per 0.5 ml sample	-	37.3% ± 4.2	253.63 ± 26.6 µg per 8.5 ml sample	-	87.57 ± 2.38
Pulford et al. 2013 [14]	93 µg per 2 ml sample	12 – 435 µg	-	-	-	-

37°C [8], up to 6 months at room temperature and additional 2 weeks at 30°C [9] or 8 months at room temperature [10]. One company, DNA Genotek (Kanata, Ontario, Canada) showed that high molecular weight DNA can be maintained even after 5 years of storage at room temperature, 6 months at 37°C or 3 months at 50°C (http://www.dnagenotek.com/US/pdf/PD-WP-005.pdf).

Manufacturers such as DNA Genotek recommend measuring DNA amounts by double strand DNA detection assays such as Picogreen (Quant-iT PioGreen® dsDNA Reagent; Lifetechnologies) or the QuantiFluor® dsDNA System (Promega) rather than by spectrometry at the 260/280 nm ratio because polysaccharides and RNA may impact the UV measurements. However, these assays measure double-stranded DNA and do not differentiate between human or foreign DNA. ABI Taqman™ assays, e.g., for the RNAse P locus, can help to determine the amount of human DNA in samples [24,25].

When comparing non-invasive methods for DNA isolation, such as sputum collection, buccal cells or foam sponge-assisted saliva/buccal cell harvest, there seems to be a consensus that saliva collection tubes are preferred for obtaining large amounts of good quality DNA [1,26]. However, obtaining large amounts of DNA from young children who are unable to produce saliva into a collection tube or from patients with dry-mouth, can be a problem. Buccal cell collection often results in low DNA yields and collection of buccal cells on cytology brushes without preservatives may enhance DNA degradation [27], however, saliva sponges or brushes in combination with commercial preservatives (saliva collection tubes) can stabilize buccal cell DNA and increase DNA yields.

Genome-wide analyses

Generally speaking, in the majority of studies no difference between saliva DNA and blood DNA was observed with regards to genome-wide studies such as SNP genotyping [13,16,17,28,29]. Both DNA sources provide sufficient amounts of DNA to perform genome-wide studies such as GWAS or exome sequencing. While typically less than 1 µg of DNA is needed for the actual analysis, genotyping facilities usually require higher amounts (e.g., 2.5 µg) to account for quality testing and possible repeats. Saliva DNA passed quality control call rate thresholds and was successfully run on different genotyping platforms. Tests with Affymetrix Human Mapping GeneChip 6.0 arrays and Taqman assays or genome-wide Illumina Beadchip arrays showed little difference between DNA from saliva and blood samples. Call rate and genotype concordance are 97% and higher for both DNA sources and platforms [13,17]. The call rate for saliva DNA appears to increase with an increasing ratio of human DNA in samples and Hu and colleagues found that samples with more than 31% human DNA content resulted in call rates of 96% or higher [24]. Another study also found a correlation between increasing amounts of human DNA in samples and improved call rates [19]. In our own experience we obtained excellent call rates above 96% with genomic saliva DNA using Illumina Linkage 24 and GoldenGate SNP genotyping arrays. There were, however, instances when the amount of double stranded DNA was insufficient to perform analyses.

Whether the performance of DNA from saliva is comparable to that from blood for the detection of Copy Number Variants (CNVs) is a controversial issue. In a small set of test samples, DNA from saliva performed as well as

Table 2. *Summary of performance characteristics.*

Performance Characteristics	Blood-derived DNA	Saliva-derived DNA
Sample storage and shipping	Yield decreases at high temperature (without stabilizing agent)	Stable at high temperature (with stabilizing agent)
Cost (collection supplies and commercial DNA isolation kits)	~ $21 (not including phlebotomy)	~ $24 (self-administration possible)
Compliance of study subjects	Less compliance, especially in children; more invasive	More compliance; 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
Genome-wide analysis	High call rate	High call rate
DNA methylation analysis	Suitable	Comparable to blood-derived DNA
PCR based analysis	Good quality	Good quality

DNA from blood for CNV detection [30]. On the other hand, CNV analyses yielded significant differences in a comparison test using blood and saliva samples from 30 individuals [29]. Another study compared 63 blood and 33 saliva samples (in duplicates) and concluded that the concordance of CNVs between duplicates was always higher in blood compared to saliva samples [6]. However, it should be noted that the saliva DNA was extracted from buccal cell rinses with mouth rinse as preservative and therefore the quality of DNA may differ from that of currently available optimized commercial saliva collection kits.

In the past, epigenetic studies were often based on genomic DNA from whole blood to compare tissue-specific methylation patterns in individuals. However, genome-wide DNA methylation patterns in saliva-derived genomic DNA appear to be relatively consistent with patterns found in DNA derived from whole blood [31] although some tissue-specific differences exist. Relatively high concordance between blood- and saliva-derived genomic DNA has also been found in several other studies, including a study on genome wide methylation patterns of sex differences [32] and a study on age-related changes of methylation patterns [33]. In a study of monozygotic twins with Feto-fetal Transfusion Syndrome (FFTS) saliva DNA was analyzed in addition to whole blood DNA in order to investigate the intra-individual methylation status of the S-COMT promoter [18]. A strong correlation between intra-individual methylation levels was detected in blood- and saliva-derived DNA.

Targeted capture of exome sequences is an increasingly popular method for genome-wide investigation of genetic variants related to disease phenotypes. Depending on the question to be asked, DNA from specific tissues or genomic DNA from blood, buccal cells or saliva are being used. We have successfully used genomic DNA from blood or from saliva for whole exome sequencing [34]. The conclusion of a recent study comparing Mendelian errors in exome sequencing data of blood, saliva and buccal cell DNA did not find differences between DNA sources. The concordance between the individual DNA sources was very high (96%) and could be increased to 99.9% after appropriate data filtering. Saliva DNA, however, produces more unmapped reads than blood DNA [35]. These unmapped reads are mostly due to sequences captured from commensal or pathogenic microbiota present in the oral cavity. Exome sequencing from saliva DNA may thus offer an interesting avenue to correlate the composition of the oral microbiome to specific disease phenotypes.

PCR-based genotyping

While it can be generally accepted that DNA extracted from blood, tissue or saliva works for simple PCR reactions, DNA quality becomes more of an issue for multiplex PCR assays. Viltrop and colleagues examined different extraction techniques for two commercially available saliva DNA extraction kits and three blood DNA extraction kits [36]. Multiplex PCR was performed using a 124-plex forensic PCR array [37]. Required DNA amounts range from 0.5 ng to 50 ng, depending on whether single primer pairs are used for PCR or whether complex multiplexing assays are being performed [37]. DNA extraction from fresh blood by traditional phenol-chloroform extraction performed best in the multiplex PCR assays and was used as a standard. Relative PCR efficiency for one of the saliva-derived DNAs (DNAGenotek) had similar efficiency as the phenol-chloroform-extracted DNA. The call rate (missing signals) was excellent for all tested kits and so was the rate for false signals (16 false signals in 11,092 calls). However, 11 of these false signals occurred in a single saliva sample of a smoker [36]. The oral environment (smoking) and collection conditions may contribute to the variability in DNA amounts and DNA quality rather than the storage condition of commercially available saliva kits. A different study, which compared storage conditions of saliva samples for extended periods of time (up to 6 months at room temperature and additional 2 weeks at 30°C) came to the conclusion that preserved saliva DNA resulted in good quality real time PCR and hotstart PCR products regardless of the storage time [9]. Other studies comparing saliva- and blood-derived DNA for PCR genotyping for Restriction Fragment Length Polymorphisms (RFLP) also found no difference in suitability of either DNA source [38]. Nunes and colleagues showed that PCR fragments from saliva DNA, even after prolonged storage of saliva, are suitable for high resolution melting curve analysis and RFLP [10].

In summary, saliva collection with commercially available kits is a relatively non-invasive method to collect large amounts of genomic DNA of sufficient quality for most genetic studies. Performance characteristics of blood and saliva DNA are summarized in *Table 2*. Commercially available saliva collection kits contain stabilizers that allow saliva to be stored at room temperature for extended periods of time. Saliva samples are the method of choice for many large genetic studies because of the ease of collection and sample handling. Saliva self-collection saves resources compared

to phlebotomy, allows access to patients who are otherwise difficult to reach and increases willingness to participate in studies. Caveats include that the quality and quantity of the DNA is more variable than in peripheral blood samples and that various amounts of bacterial and food-derived DNA may reduce call rates. Quality testing of DNA samples is therefore important.

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