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

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Abstract

In this study, we compared the response rates of blood, saliva, and buccal cell samples in a pilot study on the Danish nurse cohort and examined the quantity and quality of the purified genomic DNA. Our data show that only 31% of the requested participants delivered a blood sample, whereas 72%, 80%, and 76% delivered a saliva sample, buccal cell sample via mouth swabs, or buccal cell sample on FTA card, respectively. Analysis of purified genomic DNA by NanoDrop and agarose gel electrophoresis revealed that blood and saliva samples resulted in DNA with the best quality, whereas the DNA quality from buccal cells was low. Genotype and PCR analysis showed that DNA from 100% of the blood samples and 72% to 84% of the saliva samples could be genotyped or amplified, whereas none of the DNA from FTA cards and only 23% of the DNA from mouth swabs could be amplified and none of the DNA from swabs and 94% of the DNA from FTA cards could be genotyped. Our study shows that the response rate of self-collection saliva samples and buccal cell samples were much higher than the response rate of blood samples in our group of Danish nurses. However, only the quality of genomic DNA from saliva samples was comparable with blood samples as accessed by purity, genotyping, 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 increase the response rate considerably in epidemiologic studies. (Cancer Epidemiol Biomarkers Prev 2007;16(10):2072–6)

Introduction

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. Thus far, the preferred choice of genetic material has been blood samples because they provide large amounts of cells containing not only DNA but also a range of physiologic agents (1). 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. Several studies have found that exfoliated buccal epithelial cells are promising alternative sources of DNA (1-11). Different protocols to obtain genomic DNA have been evaluated. Some studies have found that mouthwash samples yield high amounts of high-quality genomic DNA (3, 7, 12). Other studies have compared mouthwash samples with cytobrush samples and have found that mouthwash specimens are superior to cytobrushes for obtaining high molecular weight DNA (1, 4, 6). However, the disadvantage with mouthwash samples are that donors need to swish and spit an alcohol-containing mouthwash solution, which is distasteful (9) and has been reported to cause a burning sensation in the mouth (4). Alternatively, cytobrushes have been used to brush oral mucosa, and then transferred to a card treated to inhibit bacterial growth (5). However, a review of current practices note that quantities of DNA collected on these cards have not been sufficient for spectrophotometric detection (13). 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 (14). The purpose of this pilot 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 survey response rates in a group of Danish nurses.

Received 7/11/07; revised 7/30/07; accepted 8/6/07.

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Cancer Epidemiol Biomarkers Prev 2007;16(10). October 2007

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Materials and Methods

Participants. The Danish Nurse Cohort was established in 1993, when all female members of the Danish Nurses Organization above 44 years old received a questionnaire. In 1999, the cohort was reinvestigated with additional inclusion of nurses, who had passed the age of 44 years between 1993 and 1999. In June 2006, the cohort comprised 30,508 nurses 51 years old or above. From this database, we randomly selected 300 nurses for this pilot study, all of which were representative of the cohort in terms of age (median age 61 years, range 51-91 years). Two hundred nurses were geographically representative of the cohort, and half of these were requested to deliver a saliva sample and the other half were requested to deliver buccal cell samples, either via mouth swabs or FTA cards. The remaining 100 nurses selected from the Copenhagen area were referred to the Department of Clinical Biochemistry, Rigshospitalet, Copenhagen, to deliver a blood sample.

Sample Collection and Processing. The samples were collected between May 1, 2006, and July 31, 2006. Nonresponder received a first reminder on May 23 and a second reminder on June 23. All nurses received information about the study, an informed consent form for signature, a questionnaire on general health and lifestyle issues, and either an Oragene DNA self-collection kit (DNA Genotek), a Catch-all sample collection swabs (Epicentre Biotechnologies), a sterile foam-tipped applicator and a FTA card (Whatman, Inc.) or a blood sample requisition, instruction on how to process the samples, and a prepaid return envelope. The samples were collected as follows.

Blood Samples. The subjects were referred to the Department of Clinical Biochemistry, Copenhagen, Denmark. Three-milliliter whole blood samples were venously collected and treated with sodium citrate anticoagulant. The blood samples were stored at 4°C until DNA extraction.

Saliva Samples. The subjects were asked to wash their mouth once with water and to wait at least 30 s. Then, the subjects were asked to spit in the blue container (DNA Genotek), to cap the blue container with the white lid, and finally to gently shake the sample. The Oragene saliva samples were stored at room temperature until DNA extraction.

Buccal Cells. Half of the subjects were asked to wash their mouth twice with water and to collect the cells by rolling the Catch-All sample collection swabs (Epicentre Biotechnologies) firmly inside the cheek, ~15 s on each side. The swab was air-dried for 15 min at room temperature and placed in the plastic tube. The other half of the subjects were asked to use sterile foam-tipped applicator to rub both cheeks and thereafter to transfer the cheek cells to the indicating FTA card (Whatman). The swabs and FTA cards were stored at −20°C and at room temperature, respectively, until DNA extraction.

DNA Extraction. DNA was extracted from blood samples using the E.Z.N.A. Blood DNA miniprep kit (Omega Bio-tek) as described by the manufacturer. Briefly, 2 mL whole blood sample were transferred to a 15 mL tube, 150 μL proteinase K (20 mg/mL) was added, and the sample was mixed by vortexing. Buffer BL (2.1 mL) was added and the sample was vortexed at 5 min. Then, 20 μL RNase A solution were added and the sample was incubated at 70°C for 10 min. Then, 2.2 mL isopropanol were added and the sample was mixed. The solution was added to a column, centrifuged, washed, and eluted as described by the manufacturer. The DNA was quantified and stored at −20°C until PCR analysis.

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 [10 mmol/L Tris-HCl, 0.1 mmol/L EDTA (pH 8.0)] and quantified. The DNA samples were stored at −20°C until PCR analysis.

DNA was extracted from buccal cells using the BuccalAmp DNA extraction kit (Epicentre Biotechnologies) as recommended by the manufacturer. Briefly, 500 μL QuickExtract DNA extraction solution 1.0 were added to a 2 mL Eppendorf tube; the swab was placed in the tube and rotated at least five times before the swab was pressed against the side of the tube to ensure that most of the liquid remains in the tube. The procedure was repeated with a second swab. The sample was vortexed for 10 s, incubated at 65°C for 1 min, and vortexed again for 15 s. Thereafter, the sample was incubated at 98°C for 2 min, vortexed for 15 s, and quantified. The DNA samples were stored at −20°C until PCR analysis.

For DNA extraction from FTA cards, a slice was cut using a Uni-Core puncher (3 mm). The slice was transferred to an Eppendorf tube and 200 μL FTA purification reagent (Whatman) were added and the sample was incubated for 5 min at room temperature. The reagent was removed and the wash procedure was repeated twice. Then, 200 μL TE buffer were added; the sample was incubated for 5 min at room temperature after the buffer was removed. This step was repeated once. Then, 35 μL 0.1 N NaOH, 0.3 mmol/L EDTA (pH 13.0) were added and the sample was incubated for 5 min at room temperature followed by addition of 65 μL of 0.1 mol/L Tris-HCl (pH 7.0). The sample was vortexed five times and incubated for another 10 min. Finally, the sample was vortexed 10 times, the FTA card slice was removed, and the DNA was quantified. The DNA samples were stored at −20°C until PCR analysis.

Quantification of DNA. The concentration of 1 μL DNA sample was determined using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies). The 260/280 and 260/230 nm ratios was calculated by the NanoDrop spectrophotometer and used to evaluate the DNA purity. Moreover DNA (5 μL) was loaded on a 1% agarose gel and visualized by ethidium bromide staining.
DNA from Blood, Saliva, and Buccal Cell Samples

Genotyping. Genotyping was done using TaqMan assay (Applied Biosystems). The PCR reaction was done as described by the manufacturer (Applied Biosystems). The HFE-282 primer and probe sequences were 5'-GGCC-TGGATAACCTTGGCCTGAC-3' (forward primer), 5'-GTCATGACCCAGTCAATGAG-3' (reverse primer), 5'-VIC-AGAGATATACGgcCAGGT-MGB-3' (probe 1), and 5'-6-FAM-CAGAGATATACGgt-MGB-3' (probe 2), whereas the HFE-63 primer and probes were 5'-GAAGCTTGGCCTACGATG-3' (forward primer), 5'-CATCTGCTGAAATCTACTGATGAA-3' (reverse primer), 5'-VIC-CGTGTCCATGAGATG-3' (probe 1), and 5'-6-FAM-CGTGTCCATGAGATGATG-MGB-3' (probe 2). The allele-specific fluorescence was measured using an ABI PRISM 7900HT Sequence Detector System (Applied Biosystems). Water was included as control and previously genotyped samples were included as positive controls.

PCR Amplification and Sequencing. The quality of purified genomic DNA was examined by amplification of DNA fragments routinely used in our laboratory. APC exon 2 and flanking intron sequences (242-bp fragment) was amplified using the following primers APC-ex2-F, 5'-TGTAACCAACGCGCCAGTAAATACAGATCATGCTCTGAGT-3'; APC-ex2-R, 5'-CACGAAACA-GCTATGACACACCTAAAGTGCAATTGAG-3' containing M13 primer extensions. PCR amplification was done in 50 μL containing 0.5 μmol/L primers, ~50 ng genomic DNA, 2.0 mmol/L MgCl₂, 0.2 mmol/L deoxy-nucleotide triphosphate, 5 μL Taq polymerase buffer (Promega), and 0.4 μL Taq polymerase (Promega). Thermocycling was done on an Eppendorf Mastercycler with initial denaturation at 95°C for 4 min. This was followed by six cycles each consisting of 45 s denaturation at 94°C, 45 s annealing at 62°C, and 60 s extension at 72°C. Then, 19 cycles each consisting of 30 s at 94°C, 30 s at 53°C, and 90 s at 72°C were done. The process was concluded with a final extension of 10 min at 72°C.

PCR fragments was resolved on a 1% agarose gel and visualized by ethidium bromide staining. The PCR products were finally purified using NucleoFast 96 PCR plates (Macherey-Nagel), sequenced using the BigDye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems), and analyzed on an ABI3730 DNA analyzer (Applied Biosystems).

Results

Altogether, 203 (67.7%) of the nurses returned the questionnaire. The two reminders almost doubled the response rate, but the rate varied according to DNA collection method. Table 1 shows that the highest rate (80%) was obtained among nurses who were requested to deliver buccal cells via mouth swabs, followed by buccal cells on FTA cards (76%) and saliva (72%), whereas the lowest response rate (53%) was obtained among nurses who were requested to deliver a blood test. However, the actual rate of samples obtained further reduced the response rate, because only 31 of 53 nurses delivered a blood sample, whereas all the nurses who returned a questionnaire also returned a saliva sample, a mouth swab, or a FTA card (Tables 1 and 2). In total, the highest rate of samples (40 of 50) was obtained among nurses who were requested to deliver buccal cells using Epicentre swabs, and the lowest rate (31 of 100) was obtained among the nurses requested to deliver blood samples. Of the returned samples, one Oragene container was empty upon arrival, whereas one mouth swab and two FTA cards did not contain any DNA.

The amount and purity of DNA was examined by Nanodrop analysis. The estimated amount of total DNA extracted from 2 mL blood samples varied between 11.3 and 59.6 μg with a mean of 28.4 μg, from 0.5 mL Oragene saliva samples between 0.9 and 64.2 μg with a mean of 10.8 μg, from mouth swabs between 9.1 and 194.9 μg with a mean of 64.4 μg, and from FTA cards

### Table 1. Number and proportion of returned questionnaires obtained at start, first, and second reminder according to DNA collection method

<table>
<thead>
<tr>
<th>Method of DNA collection</th>
<th>Date of start:</th>
<th>First reminder:</th>
<th>Second reminder:</th>
<th>Respondents, total, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood samples (n = 100)</td>
<td>May 1, n (%)</td>
<td>May 23, n (%)</td>
<td>June 23, n (%)</td>
<td></td>
</tr>
<tr>
<td>Blood samples (n = 100)</td>
<td>21 (21)</td>
<td>21 (21)</td>
<td>11 (11)</td>
<td>53 (53)</td>
</tr>
<tr>
<td>Blood samples (n = 100)</td>
<td>43 (43)</td>
<td>21 (21)</td>
<td>8 (8)</td>
<td>72 (72)</td>
</tr>
<tr>
<td>Blood samples (n = 100)</td>
<td>26 (52)</td>
<td>12 (24)</td>
<td>2 (4)</td>
<td>40 (80)</td>
</tr>
<tr>
<td>Blood samples (n = 100)</td>
<td>16 (32)</td>
<td>16 (32)</td>
<td>6 (12)</td>
<td>38 (76)</td>
</tr>
<tr>
<td>Participants total, N = 300</td>
<td>106 (35.3)</td>
<td>70 (23.3)</td>
<td>27 (9.0)</td>
<td>203 (67.7)</td>
</tr>
</tbody>
</table>

### Table 2. Comparison of DNA yield and quality according to DNA collection method

<table>
<thead>
<tr>
<th>Method of DNA collection</th>
<th>Blood</th>
<th>Saliva</th>
<th>Buccal cells (swabs)</th>
<th>Buccal cells (FTA cards)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total amount of samples</td>
<td>31</td>
<td>72</td>
<td>40</td>
<td>38</td>
</tr>
<tr>
<td>Failed sample</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Amount of sample used</td>
<td>2 mL</td>
<td>0.5 mL</td>
<td>2 swabs</td>
<td>One 3-mm punch</td>
</tr>
<tr>
<td>Mean DNA concentration (ng/μL)</td>
<td>56.8</td>
<td>108.0</td>
<td>128.8</td>
<td>3.6</td>
</tr>
<tr>
<td>Mean total DNA concentration (μg; range)</td>
<td>28.4 (11.3-59.5)</td>
<td>10.8 (0.9-64.2)</td>
<td>64.4 (9.1-194.9)</td>
<td>0.36 (0.09-1.33)</td>
</tr>
<tr>
<td>Mean 260/280 nm ratio (range)</td>
<td>1.79 (1.57-1.92)</td>
<td>1.63 (1.13-1.88)</td>
<td>1.15 (1.10-1.39)</td>
<td>0.91 (0.30-1.54)</td>
</tr>
<tr>
<td>Mean 260/230 nm ratio (range)</td>
<td>1.44 (1.08-2.14)</td>
<td>0.80 (0.36-1.33)</td>
<td>0.17 (0.03-0.51)</td>
<td>0.21 (0.08-0.46)</td>
</tr>
<tr>
<td>Genotyping</td>
<td>100%</td>
<td>72%</td>
<td>None</td>
<td>94%</td>
</tr>
<tr>
<td>PCR amplification</td>
<td>100%</td>
<td>84%</td>
<td>23%</td>
<td>None</td>
</tr>
</tbody>
</table>

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(one punch) between 0.09 and 1.33 μg with a mean of 0.36 μg (Table 2). The indicating circle on the FTA cards have recently been estimated to contain 100 punches (8), which would give a total mean DNA yield of ~36 μg from one FTA card.

The mean values of the 260/280 and 260/230 nm ratios were 1.79/1.44 and 1.63/0.80 for genomic DNA purified from blood and saliva, respectively, whereas the mean 260/280 and 260/230 nm ratios from DNA obtained from swabs and FTA cards were 1.15/0.17 and 0.91/0.21, respectively.

The DNA was further examined by agarose gel electrophoresis (Fig. 1). For blood and saliva samples, a visible band of high molecular weight DNA and a smear over a broad size range was observed. In contrast, no visible DNA appeared on the agarose gels for buccal cells from swabs and FTA cards.

To examine the quality of the purified DNA, the HFE-282 and HFE-63 hemochromatosis genotypes were determined by TaqMan analysis. DNA from 100% of the blood samples, DNA from 72% of the saliva samples, DNA from none of the swab samples, and DNA from 94% of the FTA card samples were genotyped (Table 2). Moreover, PCR was done with primers recognizing exon 2 of the adenomatous polyposis coli (APC) gene, which resulted in a 242-bp fragment. DNA from all blood samples (100%) resulted in one specific PCR fragment, DNA from 84% of the saliva samples resulted in one specific PCR fragment, whereas only DNA from 23% of the buccal samples from swabs were weakly amplified while DNA from none of the FTA cards were amplified. No cross-contamination or DNA degradation was observed during the PCR reactions. For blood and saliva samples, 100% and 67%, respectively, of the PCR fragments could be sequenced, whereas none of the swab PCR fragments could be sequenced.

**Discussion**

In epidemiologic population-based studies, high response rates are of utmost importance to the validity of data. The Danish Nurse Cohort Study is a large population study based on survey data. High response rates were obtained both in 1993 (86%) and in 1999 (77%). We conducted this pilot study to examine if collection of genomic DNA would influence the response rates of questionnaires and to evaluate the DNA yield and purity of genomic DNA using various collection methods.

The study showed that more than two third of the nurses returned the questionnaire. Although this response rate was 10% lower than the rate obtained in 1999, it indicates that Danish nurses are willing to donate genomic DNA. However, response rates varied considerably between DNA collection methods. In average, 75% of those requested to deliver saliva or buccal cells returned the questionnaire, and almost all of those also delivered a sample. For saliva samples, our response rate was comparable with the recently described 80% (14). However, only half of those invited to donate a blood sample answered the questionnaire and ultimately less than one third turned up to have their blood sample taken. These findings correspond well with the findings in the Nurses’ Health Study,4 and they suggest that delivery of blood implies certain barriers that are difficult to overcome. Therefore simple, self-administrated sample collection methods, including saliva and buccal cell samples, increase participation rates significantly in particular among elderly participants.

The mean DNA yield from 2 mL blood was 28.4 μg, whereas 0.5 mL Oragene saliva sample resulted in a mean DNA yield of 10.8 μg, which is lower than recently published data (14, 15). However, in these studies, DNA was purified from 2 mL Oragene saliva sample. Because the average amount of saliva sample in our study was ~4 mL (including buffer), we will be able to purify significantly larger amounts of DNA if necessary. The highest mean DNA yield was observed from buccal cell swabs. Because we were only able to visualize high molecular weight DNA from blood and saliva on agarose gels, these results suggest that the high amount of estimated DNA in the mouth swab samples is due to contamination of protein or other organic substances. The lowest DNA yield was obtained from one FTA card punch using a recommended DNA purification protocol (Whatman). Because one FTA card contains ~100 punches (8), the total amount of estimated purified DNA is comparable with the other methods.

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 and FTA cards contain chemicals, which prevents the

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4 Susan E. Hankinson, personal communication.
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 (4), whereas mouthwash samples contain 34% to 49% of human DNA (3,4). In contrast, saliva samples contained an average human DNA yield of 68% (14).

The quality of genomic DNA was examined by measurements of the 260/280 and 260/230 nm ratio by NanoDrop analysis. 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 1.79, whereas the average ratios from DNA from saliva samples were 1.63. This is comparable with the reported value from DNA Genotek and recently published data (14, 15). In contrast, the average 260/280 nm ratios for DNA from mouth swabs and FTA cards were 1.15 and 0.91, respectively, which is also comparable with recent results (15). This suggests that these samples are contaminated with proteins, which can overestimate the amount of DNA in these samples. Moreover, whereas the average 260/230 nm ratio for DNA purified from blood was 1.44, the 260/230 nm ratios for DNA from saliva, mouth swabs, and FTA cards were 0.80, 0.17, and 0.21, respectively, which is in agreement with recent results (15). These results suggest that DNA purified from mouth swabs and FTA cards are contaminated with carbohydrates, peptides, phenols, buffer salts, or other aromatic compounds, which could be undesirable for some downstream enzyme applications, including PCR.

To examine whether DNA purified from the different collection methods could be used in genotyping and mutational screening of disease causing genes, TaqMan assay, PCR, and sequencing was done. The results show that all blood samples could be genotyped, amplified, and sequenced, whereas 72% of the saliva samples could genotyped and 84% could be amplified. The genotyping result is lower than the recently published result of 96% (14) and the difference could be due to the use of different DNA extraction methods. Future studies will aim to show whether this is the case. In contrast, none of the DNA from mouth swab could be genotyped and only few could be weakly amplified, suggesting that the DNA quality highly affects genotyping and PCR amplification. Most of the DNA purified from FTA cards (94%) could be genotyped, whereas no PCR amplification was obtained, probably due to the low amount of DNA. An alternative DNA purification protocol using 6-mm punches has recently been described to increase the amount of DNA from FTA cards (16). All of the DNA from the blood and 67% of the DNA from saliva samples could moreover be sequenced. These results indicate that saliva samples—besides genotyping analysis (14, 15)—can be used in mutational screening of disease-causing genes.

Our results show that saliva samples are superior over buccal cell samples in DNA quality. Moreover, Oragene saliva samples can be stored at room temperature for up to at least 1 year. Because of the ease of collection, the convenient storage of saliva samples, the high response rate, and the high DNA quality, we suggest that saliva samples are a good alternative to blood samples in epidemiologic studies.

Acknowledgments
We thank Vibeke Trandbohus and Shehrukh Jamil for technical assistance.

References
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