Buccal Cell DNA Yield, Quality, and Collection Costs: Comparison of Methods for Large-scale Studies

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Abstract
There is considerable interest in noninvasive and cost-effective methods for obtaining DNA in large-scale studies. In this randomized crossover study of 22 participants, we compared the DNA yield, quality, and associated costs of buccal cell DNA collected using cytobrushes (three brushes per collection) and swish (i.e., mouthwash) in self-administered procedures. There was a nonstatistically significant higher yield from the mouthwash compared with cytobrush collections (15.8 µg versus 12.0 µg, respectively; P = 0.53). PCR reactions that required short (0.3 kb) or intermediate (1.1 kb) DNA fragments were 100% successful for DNA from brush and mouthwash, whereas PCRs for reactions that required long fragments (7.8 kb) failed for all of the participants from cytobrush DNA and were 81% successful for DNA from the mouthwash source. The brush collections provided sufficient DNA for an estimated 150–225 PCR reactions requiring short and intermediate DNA fragments. The estimated per person costs for buccal brush DNA collections in large studies were less then half ($8.50) those for the mouthwash method ($18). In addition, we tested whether variations in the written instructions for the cytobrush collection methods conducted via mail. Secondarily, we examined whether variations in the written instructions for the cytobrush collection could improve DNA yield, including collection in the morning before eating (versus anytime) and the addition of a step for participants to rub their cheek against their teeth for 30 s. Finally, we determined the costs associated with these two DNA collection methods. The information from this study was used to select the buccal cell collection method and procedures for the VITAL (VITamins and Lifestyle) Study, a cohort study of dietary supplements and cancer risk among 75,000 men and women ages 50–74 in western Washington.

Materials and Methods
Participants
For this randomized crossover study, we recruited 24 participants from in-house staff and their spouses who were of similar age as VITAL cohort (45 years or older). Twenty-two participants (9 men and 13 women) completed the study between June and September, 2000. One woman declined to provide a mouthwash sample. This study was approved by the Institutional Review Board at the Fred Hutchinson Cancer Research Center.

Sample Collections
Participants received three mailings: two for cytobrush collections and one for mouthwash. Each mailing was separated by 4 weeks to allow for recovery of the oral mucosa.

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2 To whom requests for reprints should be addressed, at Public Health Sciences Laboratories, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, DE320, Seattle WA 98109-1024.
Cytobrush Collection Method. Each cytobrush mailing included a cover letter, a consent form (first mailing only), one page of collection instructions with a photograph, and a sterile packet of three color-coded cytology brushes. For both brush mailings, participants were randomized to collect cells either in the morning before eating or anytime during the day. All of the participants were instructed to rinse their mouths with tap water for 10 s before collection, and to scrape their right cheek with brush one, left cheek with brush two, and right cheek again with brush three.

All of the participants collected cells using two sets of instructions (one for each mailing). “Simple counter-pressure” instructions specified to twirl the brush while moving it downward and applying counter pressure with their fingers against the external cheek. For “rubbing with counter-pressure” participants were asked to rub their cheeks against their teeth for 30 s before following the simple counter-pressure instructions.

Mouthwash Collection Method. The third mailing included a cover letter, a page of instructions for mouthwash collection, a leak-tight conical 50-ml polypropylene test tube (Corning, New York, NY) as a collection vessel, and a bottle of trial size Scope. Participants were asked to rinse their mouths with regular tap water for 10 s and to rub their cheeks against their teeth for 15 s before collection. Participants were instructed to pour enough Scope into the test tube to reach a 20-ml mark and use this to swish, vigorously, for 60 s while pressing cheeks against molars with the tips of their fingers as was shown in the photograph. The mouthwash collection could be done at any time during the day.

For all three of the mailings, participants were asked to mail the collected cells within 24 h of collection. On receipt, brushes were stored directly without additional processing in a ~80°C freezer. Mouthwash samples were centrifuged, supernatant discarded, buccal pellets washed once with 20 ml PBS, centrifuged, PBS discarded, and stored as pellets at ~80°C until analysis.

Laboratory Analysis.

DNA Extraction and Quantification. All of the samples were extracted using QIA amp mini kits (Qiagen Inc., Valencia, CA) according to the vendor instructions for buccal swabs with some modifications. Before extraction, the brush handle was cut off with wire cutters to ~25 mm from the bristle. The incubation period with protease was increased to 30 min. After incubation, the brush was transferred to a 1000-µl sterile Eppendorf tip (Fisher Scientific, Pittsburgh, PA) and centrifuged in a sterile 15-ml conical polypropylene tube to increase recovery of DNA from the brush. Also, one extra wash was added to increase the recovery of DNA from the spin columns. The final volume was 150 µl. DNA was quantified on a SpectraMax 250 microplate spectrophotometer ( Molecular Devices, Sunnyvale, CA). DNA quantity was measured against six-point standard calibration prepared from the salmon sperm DNA (Sigma Chemical, St. Louis, MO). DNA purity was assessed using the A260/280 ratio. Buccal pellets from the mouthwash samples were extracted using the same incubation and wash steps as the brush samples. However, on thawing, pellets were vigorously vortexed for 1 min and divided into two parts for extraction to assure that the spin column not be overloaded.

PCR Amplification. The quality of buccal DNA was assessed by PCR amplification of three fragments of different sizes: epoxide hydrolase exon 4 (295 bp), NAT2 (1.1 kb), and CYP2A6 (7.8 kb). PCR amplification for epoxide hydrolase and NAT2 was performed as described (9, 10). The 7.8 kb CYP2A6 fragment was amplified using the primers described by Fernandez-Salgueiro et al. (11) and the GeneAmp XL PCR kit (Applied Biosystems, Foster City, CA). Amplified fragments were separated on a 2% NuSieve (BioWhittaker Molecular Applications, Rockland, ME) agarose gel (epoxide hydrolase) or a 0.8% agarose (Life Technologies, Inc., Rockville, MD) gel.

Data Analysis

For the analysis of the DNA yield from the brushes, we first tested for differences between the three brushes, adjusted for set of instructions, mailing, and time of collection using ANOVA for a two-period crossover design. After determining that there were no consistent differences among brushes, we computed the total DNA yield from the three brushes, which was our primary outcome variable. Analysis for differences between sets of instructions, mailings, and time of collection was performed using ANOVA for the two-period crossover design. As a consequence of the study design, the comparisons between sets of instructions and mailings were done within person (22 pairs of observations) whereas the comparison between times of collection was between persons (11 individuals in the morning group versus 11 in the anytime group). Parameter estimates for any one factor are adjusted for the other two factors.

We evaluated differences in DNA yield between brush and the swish methods by comparing the total yield of DNA from the three brushes, averaged across the two brushing occasions, to the total DNA yield from the swish method by a paired t test. We also compared the proportions of the brush and swish collections that yielded samples that could be successfully amplified for specific DNA fragments using Fisher’s exact test.

Cost Analysis

The costs of obtaining DNA from cytobrush and mouthwash methods per participant were based on actual charges for personnel time, equipment, and supplies from studies conducted at Fred Hutchinson Cancer Research Center. Personnel time includes costs for tracking returned samples and processing samples for long-term storage. Other costs include collection kits, kit assembly, other mailed materials (approach letters, instructions, consent forms, bubble wrap, absorbent cloth, and containers), postage, storage supplies, full cost of purchasing freezers and 10 years of maintenance, sample handling, and processing.

Results

Table 1 gives DNA yields by variations in the cytobrush method. We found no consistent differences in DNA yields between brushes (1, 2, and 3), by set of instructions (simple counter pressure or rubbing with counter pressure), or by time of collection (morning or anytime; all P > 0.05). Surprisingly, we obtained as much DNA from a repeat right cheek brushing (brush 3) as from the initial brushings of right and left cheeks (brushes 1 and 2): 4.1, 3.7, and 4.2 µg for brushes 1, 2, and 3, respectively. Because there was no difference between the brushes, we combined results from three brushes for each participant for additional analyses. There was also no difference in DNA yield between two cytobrush mailings, which suggests that 1 month between mailings was sufficient for recovery of oral mucosa. We found no difference in DNA yield obtained from buccal cells from the three cytobrushes collected in the morning before eating (12.0 µg) compared with those collected anytime during the day (11.9 µg), nor was there any improvement in DNA yield by implementing cheek rubbing to loosen
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4.4 as part of quality control (data not shown).

Successful from leukocyte DNA that was run in the same batch mouthwash source. The PCR amplification was 91% (20 of 22) required 7.8 kb and was 81% successful for DNA from the of the participants from cytobrush DNA for the reaction that intermediate DNA fragments. However, the PCRs failed for all DNA from brush and mouthwash that required the short or

CYP2A6 that require 0.3, 1.1, and 7.8 kb DNA fragments, 

EHX hydrolase (Ex 4), N-acetyl transferase (NAT2), and CYP2A6 that require 0.3, 1.1, and 7.8 kb DNA fragments, respectively. The PCR reactions were 100% successful for

Table 1  Comparison of total DNA yields from cytobrushes by brush number, mailing, set of instructions, and time of collection

<table>
<thead>
<tr>
<th>Cytobrush Comparisons</th>
<th>N Observations</th>
<th>µg DNA</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brush Number (comparison between brushes)</td>
<td>44</td>
<td>4.1</td>
<td>2.4</td>
</tr>
<tr>
<td>1-right cheek</td>
<td>44</td>
<td>3.7</td>
<td>1.9</td>
</tr>
<tr>
<td>2-left cheek</td>
<td>44</td>
<td>4.2</td>
<td>2.4</td>
</tr>
<tr>
<td>3-repeat right cheek</td>
<td>44</td>
<td>6.6</td>
<td>4.7</td>
</tr>
<tr>
<td>Mailing (comparison within persons)</td>
<td>22</td>
<td>12.5</td>
<td>0.39</td>
</tr>
<tr>
<td>First (baseline)</td>
<td>22</td>
<td>11.1</td>
<td>0.90</td>
</tr>
<tr>
<td>Second (4 weeks)</td>
<td>22</td>
<td>11.9</td>
<td>0.95</td>
</tr>
<tr>
<td>Procedures (comparison within persons)</td>
<td>22</td>
<td>11.9</td>
<td>0.95</td>
</tr>
<tr>
<td>Simple counter pressure</td>
<td>22</td>
<td>11.9</td>
<td>0.95</td>
</tr>
<tr>
<td>Rubbing plus counter pressure</td>
<td>22</td>
<td>11.9</td>
<td>0.95</td>
</tr>
<tr>
<td>Time (comparison between persons)</td>
<td>11</td>
<td>12.0</td>
<td>0.95</td>
</tr>
<tr>
<td>Morning</td>
<td>11</td>
<td>12.0</td>
<td>0.95</td>
</tr>
<tr>
<td>Anytime</td>
<td>11</td>
<td>11.9</td>
<td>0.95</td>
</tr>
</tbody>
</table>

a Mean per brush, where each participant had two brushes each for brush 1, 2, and 3.

b Total DNA summed across three brushes per person.

c Total DNA summed across three brushes per person, averaged > two mailings.

d One participant did not provide mouthwash.

cells (simple counter pressure = 11.9 µg and rubbing with counter pressure = 12.1 µg).

As shown in Table 2, we observed, on average, 30% higher DNA yields with mouthwash than with the brush method (15.8 versus 12.0 µg), although the difference was not statistically significant. However, the range of DNA yield was greater with mouthwash than with cytobrush (1.8–49.7 versus 4.4–27.1 µg) as was the SD (11.3 versus 5.8 µg) for comparison of mouthwash versus mean of three brushes. For cytobrush mailings, the interindividual variation was much greater than the intraintividual variation (17.3 versus 2.3 µg, data not shown). We could not measure the intraintividual variation for the swish because we only collected one swish sample per participant. The DNA purity, as assessed by the A260:A280 ratios, was similar in the mean and range for the two collection methods (Table 2).

Because the main purpose of collecting DNA was for future genotyping studies, we determined the PCR amplification success rate for three genes that require different length PCR fragments. Specifically, we performed PCR on epoxide hydrolase (EHX Ex 4), N-acetyl transferase (NAT2), and CYP2A6 that require 0.3, 1.1, and 7.8 kb DNA fragments, respectively. The PCR reactions were 100% successful for DNA from brush and mouthwash that required the short or intermediate DNA fragments. However, the PCRs failed for all of the participants from cytobrush DNA for the reaction that required 7.8 kb and was 81% successful for DNA from the mouthwash source. The PCR amplification was 91% (20 of 22) successful from leukocyte DNA that was run in the same batch as part of quality control (data not shown).

Table 3 gives cost comparisons of buccal cell collection via the mail using the cytobrush versus the mouthwash method. These figures assume the economies of a large-scale study (i.e., 75,000 mailings) and give costs for a 100% response rate and a more realistic 50% response rate, as realistic upper and lower bands of expected response rates. The cytobrush method costs about half as much as the mouthwash method ($8 versus $18, at 50% response).

Discussion

In this randomized crossover study, we did not find statistically significant differences in DNA yields from three cytobrushes (collected at one time) as compared with one mouthwash collection when collections were obtained via mail. Our results conflict with those reported by other investigators, primarily because our mouthwash DNA yields were lower and cytobrush DNA yields were higher than those reported previously (2, 3, 6). Unlike preceding reports, we measured mouthwash and cytobrush DNA yields in the same participant, and we allowed 4 weeks for the recovery of the oral mucosa between collections. This is important as there appears to be a wide variation between individuals in the desquamation of oral mucosa (12). Our comparisons of DNA yields between cytobrushes and mouthwash might be misleading if specimens from cytobrushes contain significantly more bacterial DNA than those from mouthwash. It has been shown that substantial amount of DNA from oral sources can be of microbial origin (4, 5). Because human DNA assays are difficult and expensive, in our study we quantified total DNA. To minimize food and microbial con-
Unpublished observations.

higher from the mouthwash versus systematically tested these variations in methods. To our knowledge, other studies have not optimized DNA yield from the cytobrush method. We found that for 150\(\text{ samples were processed immediately and frozen at } -80^\circ\text{C until DNA extraction to additionally prevent bacterial growth and preserve the quality of DNA. Feigelson et al. (5) have shown that brushing teeth before mouthwash collection decreased DNA yields by 40%. Thus, it is possible that the rinsing step in our study reduced DNA yields from mouthwash collections. A final possibility as to why our DNA yields were more similar between the two methods then in other studies is that we optimized our DNA extraction from cytobrushes.

The DNA quality, as measured by PCR amplification success, indicated that cytobrush collections contained DNA fragments sufficient for short and intermediate amplification primers (up to 1.1 kb), with poor results for longer gene fragments (i.e., 7.8 kb), possibly because of more significant degradation of cytobrush DNA as seen on agarose gels (data not shown). The degradation of DNA from cytobrush suggests that the time at ambient temperature may be an issue. However, in our pilot work,\(^3\) we found no difference in PCR success based on ambient temperature (up to 5 days) or storage at \(-70^\circ\text{C for up to 3 years). Our findings support previous studies (3, 4), which have reported similar results in PCR success for fragments up to 1.5 kb (4). Furthermore, it is estimated that for the vast majority of polymorphisms (99%), PCR amplification reactions do not require longer than 1 kb DNA fragments.

Another indicator of the quality and quantity of the human DNA is the number of PCR reactions that can be conducted per individual specimen. Given that each amplification requires only 2–3 \(\mu\text{l of the DNA extract per PCR reaction, DNA collection from three cytobrushes would provide enough DNA for 150–225 PCRs.}

A secondary aim of this study was to refine instructions for optimizing DNA yield from the cytobrush method. We found that additional cheek rubbing to loosen cells was not needed before scraping with the cytobrush. Brushings restricted to the morning before eating (as opposed to anytime of day) did not yield a greater quantity, and additional DNA recovery was possible from the repeat cheek brushing. To our knowledge, other studies have not systematically tested these variations in methods.

In summary, we found that total DNA yield was 30% higher from the mouthwash versus cytobrush buccal cell collection method. However, three cytobrushes should provide sufficient DNA for 150–225 PCRs. PCR success was similar for cytobrush and mouthwash buccal cell collection methods except for those amplifications that require large DNA fragments. The difference in costs between the two methods was approximately $10. Whereas these savings might not be important for small studies, large-scale studies could save hundreds of thousands of dollars by choosing the cytobrush method. We conclude that the cytobrush is a feasible and cost-effective method for obtaining genomic DNA for large-scale epidemiological studies.

Acknowledgments

We thank Lilik Zakarian for technical assistance in buccal cell DNA extractions and Melissa Mouton for acquiring the cost estimates.

References

Corrections


We wish to call attention to an error in the units given for isoflavone intakes and in the estimation of weekly isoflavone intakes according to the diet recall. The units for isoflavone intakes determined using both assessment methods should be in amol, not nmol (Table 2, page 257; Table 4, page 259; Figure 1, page 259; and in the text of the methods and results). The last sentence of paragraph two on page 255 should read “Weekly isoflavone intakes according to the diet recall were estimated as mean intake over the 2 days x 7”, and the data in Table 2 column 5 (“Estimated weekly intake”), page 257, should be as follows: “Soy consumers—Genistein 208.1 [183.6–235.8], range 13.9–5270.4; Daidzein 155.3 [137.0–176.0], range 9.2–3898.4. All women—Genistein 2.1 [1.4–2.9], range 0–5270.4; Daidzein 1.9 [1.3–2.6], range 0–3898.4.” These data were not used in any subsequent calculations and this change does not alter the discussion or conclusions.


1. Note the following sentence in line 4 of paragraph 3 of the Introduction on page 640: “. . . furthermore, C580T polymorphism co-segregates with the C74A polymorphism of intron 1 of CDKN2B (18).” should read as “. . . furthermore, C540G polymorphism co-segregates with the C74A polymorphism of intron 1 of CDKN2B (18).”

(Note: The change is C580T to C540G)

2. Note the following sentence in paragraph 2 of the Materials and Methods on page 641: “For PCR, the previously described primers (17) were used: 5’-GCC TGT TTT CTT TCT GCC CTC TG-3’ (sense). . . .” should read as “For PCR, the previously described primers (18) were used: 5’-GCC TGT TTT CTT TCT GCC CTC TG-3’ (sense; confirmed by the reported sequences: accession number U12820). . . .”

(Note: The changes are 17 to 18 and the addition of “confirmed by the reported sequences: accession number U12820.”)

We believe the major two important findings in our paper are not affected in any way by these two misquotes.

And please note the correct author names to an article in the October Issue:

Buccal Cell DNA Yield, Quality, and Collection Costs: Comparison of Methods for Large-scale Studies
Irena B. King, Jessie Satia-Abouta, Mark D. Thornquist, Jeannette Bigler, Ruth E. Patterson, Alan R. Kristal, Ann L. Shattuck, John D. Potter, and Emily White. . . . . . . . 1130
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