Short Communication

Buccal Cell DNA Yield, Quality, and Collection Costs: Comparison of Methods for Large-scale Studies[1]

Satia-Abouta, Irena B. King[2], Jessie Satia Abouta, Mark D. Thornquist, Jeannette Bigler, Ruth E. Patterson, Alan R. Kristal, Ann L. Shattuck, John D. Potter, and Emily White

Cancer Prevention Research Program, Fred Hutchinson Cancer Research Center, Seattle, Washington, 98109

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 cytobrush instructions to rub cheeks before collection or collect cells only in the morning increased DNA yield and whether repeat brushings of the same cheek reduced DNA yield. These variations resulted in no significant differences in DNA yields. We conclude that the collection of DNA with cytobrushes using simple instructions is cost effective in large-scale studies, and yields sufficient quantity and quality of DNA for genotyping.

Introduction

Given the increasing emphasis on the role of genetics in cancer development and prevention, simple and cost-effective methods are needed to obtain DNA for large-scale studies. Although the source of DNA for epidemiological studies often comes from blood collections, this method is too invasive for some participants and is expensive for large-scale studies. Therefore, various means of collecting buccal cells have become an attractive approach for obtaining DNA (1–4). The methods for collecting buccal cells are of two types: dry procedures that use a cytobrush or other implements for scraping of the oral mucosa, and wet procedures that involve swishing liquids in the mouth and spitting into a collection vessel. There are advantages and disadvantages associated with each type of collection. The advantages of the swish method appear to be higher average DNA yields, longer DNA fragments, and possibly higher percentages of human DNA (4, 5). However, the swish method requires liquid handling and centrifugation steps during sample processing, which result in increased costs for materials, mailings, and processing before freezing samples. The advantages of dry cheek collection, such as the cytobrush method, are simple assembly for large-scale mailings, light-weight postage, and efficient and cost-effective processing for long-term archiving. Several investigators described successful cheek cell scrapings and mouthwash collections for PCR-type applications (1–8). However, we know of no studies that have compared both collection methods in the same individuals.

The primary objective of this study was to compare DNA yield and DNA quality for PCR applications from dry (*i.e.*, cytobrush) compared with wet (*i.e.*, mouthwash) buccal cell 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.

Received 11/30/01; revised 4/23/02; accepted 5/30/02.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was supported by Grant R01 CA74846.
[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 anytime 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-Salguero *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

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

Cytobrush Comparisons	N Observations	μg DNA		*P*
		Mean	SD	
Brush Number (comparison between brushes)[a]				
1-right cheek	44	4.1	2.4	0.26
2-left cheek	44	3.7	1.9	
3-repeat right cheek	44	4.2	2.4	
Mailings (comparison within persons)[b]				
First (baseline)	22	12.5	6.6	0.39
Second (4 weeks)	22	11.5	4.7	
Procedures (comparison within persons)[b]				
Simple counter pressure	22	11.9	5.1	0.90
Rubbing plus counter pressure	22	12.1	6.3	
Time (comparison between persons)[c]				
Morning	11	12.0	5.1	0.95
Anytime	11	11.9	5.1	

[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.

Table 2 DNA yields and quality based on UV absorbance and PCR success rate

Collection Methods	*n*	μg DNA				A260:A280 ratio[a]		PCR success rate		
								EHX Ex 4	*NAT2*	*CYP2A6*
		Mean	SD	*P*	Range	Mean	Range	4 0.3kb	1.1kb	7.8kb
Brush	21	11.95[b]	5.8[c]		4.4–27.1	1.8	1.6–2.0	100%	100%	0%
Mouthwash[d]	21	15.8	11.26		1.8–49.7	1.9	1.6–2.0	100%	100%	81%
Difference		−3.8		0.53						

[a] UV absorbance measurements in microplates.
[b] Total DNA summed across three brushes per person, averaged over two mailings.
[c] SD over individuals and mailings of the total DNA summed across three brushes.
[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 intraindividual variation (17.3 *versus* 2.3 μg, data not shown). We could not measure the intraindividual 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-

Table 3 Buccal cell collection costs[a] per participant for cytobrush and mouthwash

Item	Cytobrush		Mouthwash	
	50% response rate	100% response rate	50% response rate	100% response rate
DNA collection kit (brush or swish)	$3.00	$1.50	$3.18	$1.59
Mailing materials including container	$1.16	$0.58	$2.24	$1.12
Assembly	$0.72	$0.36	$2.92	$1.46
Postage including return	$1.13	$0.99	$3.24	$2.74
Processing and handling	$0.80	$0.80	$4.76	$4.76
Storage supplies	$0.09	$0.09	$1.12	$1.12
Freezers and 10-year maintenance	$1.52	$1.52	$0.61	$0.61
Total	$8.42	$5.84	$18.07	$13.40

[a] Costs for large-scale studies (based on 75,000 mailings).

tamination, we instructed participants to rinse with tap water for 10 s before all of the collections and to mail samples within 24 h of collections. Additionally, on receipt at the laboratory, all of the samples were processed immediately and frozen at −80°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°C (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 μl 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.

[3] Unpublished observations.

Acknowledgments

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

References

1. Richards, B., Skoletsky, J., Shruber, A. P., Balfour, R., Stern, R. C., Dorkin, H. L., Parad, R. B., Witt, D., and Klinger, K. W. Multiplex PCR amplification from the CFTR gene using DNA prepared from buccal brushes/swabs. Hum. Mol. Genet., *2:* 159–163, 1993.

2. Le Marchand, L., Lum-Jones, A., Saltzman, B., Visaya, V., Nomura, A., and Kolonel, L. N. Feasibility of collecting buccal cell DNA by mail in a cohort study. Cancer Epidemiol. Biomark. Prev., *10:* 701–703, 2001.

3. Garcia-Closas, M., Egan, K. M., Abruzzo, J., Newcomb, P. A., *et al.* Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. Cancer Epidemiol. Biomark Prev., *10:* 687–696, 2001.

4. Heath, E. M., Morken, N. W., Campbell, K. A., Tkach, D., Boyd, E. A., and Strom, D. A. Use of buccal cells collected in a mouthwash as a source of DNA for clinical testing. Arch. Pathol. Lab. Med., *125:* 127–133, 2001.

5. Feigelson, H. S., Calle, E. E., Rodriquez, C., Jacobs, E. J., Robertson, A. S., and Thun, M. J. Determinants of DNA yield from buccal cell samples collected with mouthwash. Cancer Epidemiol. Biomark. Prev., *10:* 1005–1008, 2001.

6. Lum, A., and Le Marchand, L. A simple mouthwash method for obtaining genomic DNA in molecular epidemiological studies. Cancer Epidemiol. Biomark. Prev., *7:* 719–724, 1998.

7. de Vries, H. G., Collee, J. M., van Velduizen, M. H., Achterhof, L., Smit Sibinga, C. T., Scheffer, H., Buys, C. H., and ten Kate, L. P. Validation of the determination of δF508 mutations of the cystic fibrosis gene in over 11 000 mouthwashes. Hum. Genet., *97:* 334–336, 1996.

8. Zheng, S., Ma, X., Buffler, P. A., Smith, M. T., and Wiencke, J. K. Whole genome amplification increases the efficiency and validity of buccal cell genotyping in pediatric populations. Cancer Epidemiol. Biomark. Prev., *10:* 697–700, 2001.

9. Ulrich, C. M., Bigler, J., Whitton, J., Bostick, R. M., Fosdick, L., and Potter, J. D. Epoxide Hydrolase Tyr113His polymorphism is associated with elevated risk of colorectal polyps in the presence of smoking and high meat intake. Cancer Epidemiol. Biomark. Prev., *10:* 875–882, 2001.

10. Bigler, J., Chen, C., and Potter, J. D. Determination of human NAT2 acetylator genotype by oligonucleotide ligation assay. Biotechniques, *22:* 682–690, 1997.

11. Fernandez-Salguero, P., Hoffman, S. M. G., Cholerton, S., Mohrenweiser, H., Raunio, H., Rautio, A., Pelkonen, O., Huang, J-D., Evans, W. E., Idle, J. R., and Gonzalez, F. J. A genetic polymorphism in coumarin 7-hydroxylation: sequence of the human CYP2A genes and identification of variant CYP2A6 alleles. Am. J. Hum. Genet., *57:* 651–660, 1995.

12. Ten Cate, A. R. Oral Histology Development, Structure, and Function, 3rd Edition. St. Louis, MO: The C. V. Mosby Company, 1989.