Short Communication

Determinants of DNA Yield and Quality from Buccal Cell Samples Collected with Mouthwash

Heather Spencer Feigelson,[1] Carmen Rodriguez, Andrea S. Robertson, Eric J. Jacobs, Eugenia E. Calle, Yvonne A. Reid, and Michael J. Thun

Department of Epidemiology and Surveillance Research, American Cancer Society, National Home Office, Atlanta, Georgia 30329-4251 [H. S. F., C. R., A. S. R., E. J. J., E. E. C., M. J. T.], and American Type Culture Collection, Manassas, Virginia 20110-2209 [Y. A. R.]

Abstract

Buccal cells are becoming an important source of genomic DNA in epidemiological studies, but little is known about the effect of different sampling conditions on DNA quality and yield. We used a mouthwash protocol to collect six daily buccal cell samples from 35 healthy volunteers. Twenty-four individuals (six men and 18 women) correctly completed the protocol and were included in paired analyses to determine whether "swish" time (30 s *versus* 60 s), toothbrushing before collection, or lag time between collection and DNA extraction (1 day *versus* 5, 10, or 30 days at room temperature) would affect sample quality and yield. Total DNA, human-specific DNA (hDNA), degradation of DNA, and ability to amplify by PCR were determined. hDNA yield did not significantly vary by "swish" time. However, toothbrushing 1 h before sample collection reduced the amount of hDNA by nearly 40% (34 μg *versus* 21 μg; $P = 0.06$). Median hDNA yields for samples that were held for 1, 5, 10, and 30 days before extraction were 32 μg (range, 4–196), 32 μg (2–194), 23 μg (3–80), and 21 μg (5–56), respectively. The 10- and 30-day samples had significantly less hDNA than those processed after 1 day ($P = 0.01$). PCR success rates for β-globin gene fragments of length 268 bp, 536 bp, and 989 bp were 94% or better, and high molecular weight DNA (>23 kb) was found in all but one sample. These results suggest that buccal cells should be collected before brushing teeth and processed within 5 days of collection to maximize hDNA yield.

Introduction

The preferred way to obtain genomic DNA for epidemiological study is from peripheral blood. Such samples typically yield at least 30 μg of DNA/ml of whole blood (1). Recently, epidemiological investigators have started to collect buccal cells as an alternative or supplement to peripheral blood. In contrast to blood, buccal cells offer a noninvasive and more easily collected source of DNA. Several methods of buccal cell collection have been described, including the use of treated "Guthrie" type cards, cytobrushes, cotton swabs, saline rinses, and mouthwash (1–9). Recent studies (4, 9, 10) have shown that collection using mouthwash gives greater yields than other collection methods and is feasible for use in cohort studies. However, there are still some sample collection conditions that may affect the amount and quality of hDNA[2] that remain untested. Determining the optimal conditions was important to us because we intended to use a mouthwash collection protocol to collect buccal cell samples through regular United States mail from a large, elderly cohort dispersed in 21 states in the United States (the American Cancer Society Cancer Prevention Study II cohort).[3]

We conducted a pilot study to determine how to maximize the amount and quality of hDNA that could be collected from buccal cells using a mouthwash collection protocol. Specifically, the purpose of this study was to determine whether "swish time," toothbrushing, or lag time between collection and DNA extraction would alter the amount or quality of hDNA obtained from mouthwash samples.

Materials and Methods

Sample Collection. Study participants were recruited from the American Cancer Society National Home Office Research Department. After obtaining written informed consent, participants were given data collection forms and supplies to collect six buccal cell samples over the course of two consecutive weeks (three samples each week). Participants were given labeled collection cups and instructed to pour 10 ml of Scope mouthwash into the collection cup, vigorously rinse their mouth with the Scope for a specified period of time, and then spit the mouthwash back into the cup. On the data collection forms, participants were asked to record the date and time of collection and to watch the clock to record their "swish" time for each sample. Participants were also asked to record any discomfort or difficulties in providing the sample (burning, gagging, and so forth). Buccal cell samples were collected each day by 10 a.m. and then shipped overnight to the laboratory (American Type Culture Collection, Manassas, VA) for processing. The samples were mailed in styrofoam shipping boxes but were not cooled.

As illustrated in Fig. 1, the collection procedure varied slightly on each of the six days. On day 1, samples were collected before brushing teeth with a swish time of 60 s. On day 2, samples were collected before brushing teeth with a

Received 2/23/01; revised 6/22/01; accepted 7/2/01.
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] To whom requests for reprints should addressed, at American Cancer Society, Department of Epidemiology and Surveillance Research, 1599 Clifton Road, NE, Atlanta, GA 30329-4251. Phone: (404) 929-6815; Fax: (404) 327-6450; E-mail: heather.feigelson@cancer.org.

[2] The abbreviation used is: hDNA, human-specific DNA.

[3] E. E. Calle, C. Rodriguez, E. J. Jacobs, M. L. Almon, A. Chao, M. L. McCullough, H. S. Feigelson, and M. J. Thun. The American Cancer Society nutrition cohort: rationale, study design and baseline characteristics. Cancer (Phila.), submitted for publication.

Downloaded from cebp.aacrjournals.org on January 21, 2022. © 2001 American Association for Cancer Research.

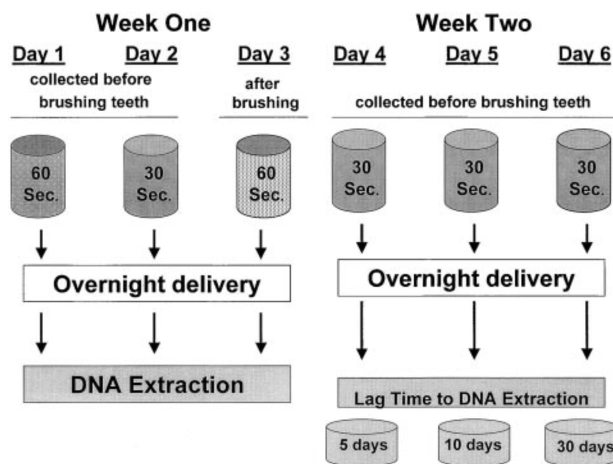

Fig. 1. Diagram of study design illustrating sample collection conditions for each of the six buccal cell samples. Samples were collected before or after toothbrushing, using a swish time of 30 s or 60 s. DNA extraction for week 1 samples was initiated on the morning after sample collection. Week 2 samples were held for 5, 10, or 30 days in the dark at room temperature before DNA extraction.

swish time of 30 s. On day 3, samples were collected 1 h after brushing teeth with a swish time of 60 s. DNA extraction was performed on all of the week 1 samples the day after the samples were collected.

In week 2, we wanted to simulate possible delays encountered when mailing samples and test how lag time to processing would affect DNA quality and yield. Our *a priori* hypothesis was that a 60-s swish would provide a higher DNA yield compared with a 30-s swish. However, we were concerned that some of our participants would be unable or unwilling to rinse their mouth with the mouthwash for as long as 60 s. Thus, we used a 30-s swish before brushing teeth for all of the week 2 samples. To test the effect of lag time, day 4 samples were held for 5 days before DNA extraction, day 5 samples were held for 10 days, and day 6 samples were held for 30 days. All of the samples were stored in the dark at room temperature before processing.

Samples were anonymized for all of the testing, and the Emory University Human Investigations Committee approved the study.

DNA Isolation. DNA was extracted from buccal cells according to a modification of the Puregene DNA Isolation method for DNA Isolation from Buccal Cells in Mouthwash (Gentra Systems, Inc.). Buccal cells collected in Scope mouthwash were pelleted and lysed in 3.0 ml of cell lysis solution. The crude DNA extract was deproteinated with 300 μg of proteinase K for 2 h at 55°C, and an additional 300 μg of proteinase K were added for overnight digestion. RNA was digested by adding 60 μg of RNase A for 30 min at 37°C. Protein from the DNA extract was removed with 1.0 ml of protein precipitation solution after centrifuging at 2000 $\times$ *g* for 10 min. DNA was then precipitated in the presence of 100 μg of glycogen and 3.0 ml of isopropanol. The DNA pellet was collected after centrifugation at 2000 $\times$ *g* for 10 min, washed in 70% ethanol, briefly air-dried, and resuspended in TLE (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.4) buffer.

DNA Yield. Total DNA yield was measured by standard UV absorbance at 260 nm. hDNA yield for each sample was determined by hybridization to the D17Z1 sequence (a primate-specific probe) using the ACES 2.0+ Human DNA Quantitation System (Life Technologies, Inc., Grand Island, NY). DNA samples were serially diluted (1:1, 1:2, 1:4, 1:8, and 1:16) and spotted onto a charged membrane. The amount of hDNA was quantified using image analysis by comparing the intensity of the hybridization signals with DNA standards of known concentrations.

DNA Integrity. To ascertain the presence of high molecular weight DNA in the buccal cell samples, isolated DNA was electrophoresed on a 0.4% agarose gel at 2V/cm in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.3) and stained with ethidium bromide. DNA degradation was shown by fragmentation of the buccal cell DNA samples compared against a known molecular weight marker (Ready-load λ DNA/*Hin*dIII fragments; Life Technologies, Inc.) with visible bands of lengths 23,130 bp, 9,416 bp, 6,557 bp, 4,361 bp, 2,322 bp, 2,027 bp, and 564 bp.

DNA integrity was also determined by PCR analysis. Three regions of the human β-globin gene (fragments of length, 268, 536, and 989 bp) were amplified as described previously (11). The 268-bp amplified products were separated in 4% agarose and stained with SYBR Green I. The 536-bp and 989-bp amplified products were separated in 3% agarose and also stained with SYBR Green I.

Statistical Analysis. Pairwise comparisons were made to determine differences in median total DNA and hDNA yield under different sample collection and storage conditions using the sign rank test. Two-sided p-values were computed. The day-1 sample (swished for 60 s, before brushing teeth) was used as the reference for sample days 2 and 3, whereas the day-2 sample (swished for 30 s, before brushing teeth) was used as the reference for the samples collected in week 2.

Results

Thirty-five volunteers were enrolled in the study. The median age of participants was 33 years (range, 23–63). Twenty-four (six men and 18 women) of them correctly completed the protocol and were included in the paired analyses of DNA yield. Four people were excluded from the paired analysis because of missing samples, and seven were excluded because they had an incorrect swish time for at least one sample. For the assessment of DNA integrity, all of the collected samples were evaluated ($n = 206$).

Table 1 shows total DNA (as estimated by spectrophotometry) and hDNA (as estimated by hybridization) yields under various sample collection conditions. Fig. 2 displays the distribution of hDNA yield (μg) for each of six sample collection conditions. hDNA yield did not vary significantly by swish time. The median amount of hDNA in the sample swished for 60 s and extracted within 48 h (sample 1) was 34 μg (range, 4–189) compared with 32 μg (4–196) for a 30-s swish ($P = 0.68$). Toothbrushing 1 h before sample collection reduced the amount of hDNA by nearly 40% (34 μg *versus* 21 μg; $P = 0.06$). The hDNA yields for samples that were held for 5, 10, and 30 days before extraction were 32 μg (2–194), 23 μg (3–80), and 21 μg (5–56), respectively. There was no difference in hDNA yield between samples that were held for 5 days compared with samples that were processed within 2 days ($P = 0.93$). However, samples held for 10 and 30 days had significantly less hDNA than sample 2 ($P = 0.01$).

Amplification by PCR of three fragments of the β-globin gene (268 bp, 536 bp, and 989 bp in length) was attempted on all of the samples collected ($n = 206$). The 268-bp and 536-bp fragments were successfully amplified on the first PCR attempt

Downloaded from cebp.aacrjournals.org on January 21, 2022. © 2001 American Association for Cancer Research.

Table 1 DNA yield from mouthwash buccal cell collection under various conditions

	Spectrophotometry Total DNA (μg)			Hybridization Human DNA[a] (μg)		
	Median	Range	P^b	Median	Range	P^b
Week #1						
Day 1						
60-s swish, before brushing	101	8–589		34	4–189	
Day 2[c]						
30-s swish, before brushing	74	19–342	0.02	32	4–196	0.68
Day 3[c]						
60-s swish, after brushing	41	10–158	<0.0001	21	1–218	0.06
Week #2[d]						
Day 4						
Stored for 5 days	85	9–514	0.50	32	2–194	0.93
Day 5						
Stored for 10 days	78	13–335	0.22	23	3–80	0.01
Day 6						
Stored for 30 days	65	11–413	0.16	21	5–56	0.01

[a] Human DNA as estimated by dot-blot hybridization.
[b] Two-sided P for pair-wise differences using sign rank test.
[c] Median differences were compared with the day 1 sample.
[d] All of the week #2 samples used a 30-s swish collected before toothbrushing; median differences were compared with the day 2 sample.

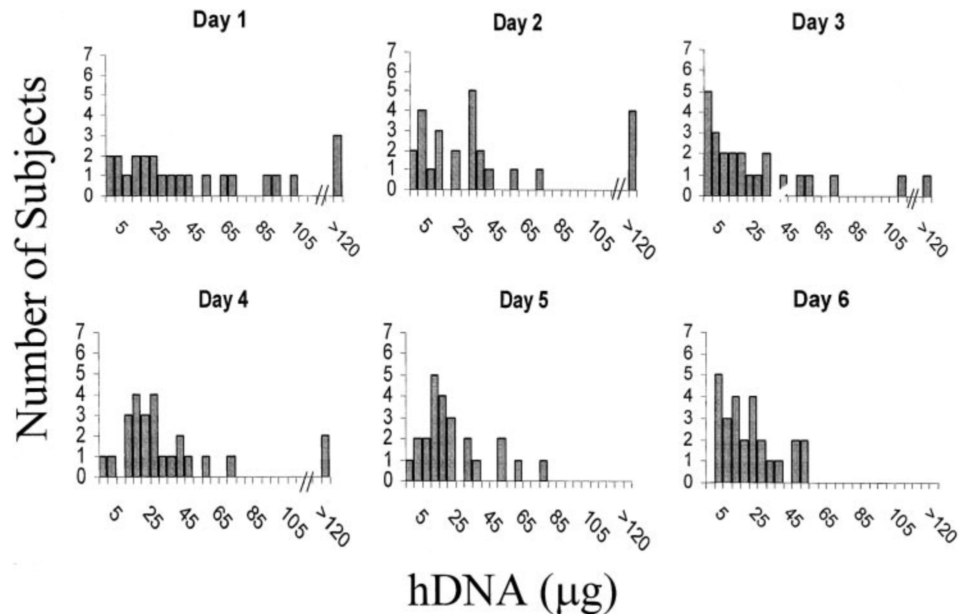

Fig. 2. Distribution of hDNA concentration (μg) as determined by hybridization. Day 1 samples were collected before brushing teeth with a swish time of 60 s. Day 2 samples were collected before brushing teeth with a swish time of 30 s. Day 3 samples were collected 1 h after brushing teeth with a swish time of 60 s. DNA extraction was performed on day 1-day 3 samples on the day after the samples were collected. Day 4-day 6 samples were all collected before brushing teeth with a 30-s swish time. Day 4 samples were held for 5 days before DNA extraction. Day 5 samples were held for 10 days, and day 6 samples were held for 30 days.

in every sample. The 989-bp fragment was successfully amplified in 187 samples on the first PCR attempt and an additional seven samples on the second PCR attempt. In 12 samples (5.8%), the 989-bp fragment could not be amplified in two PCR attempts. All of the 104 samples isolated within 48 h were successfully amplified. Of the samples stored for 5, 10, and 30 days before DNA extraction, 6 of 33, 3 of 35, and 3 of 33 failed to amplify, respectively.

Discussion

The results of this study suggest that buccal cell collection using mouthwash provides a substantial amount of high quality DNA. To maximize yield, buccal cells should be collected before brushing teeth. The amount of hDNA was nearly 40% greater ($P = 0.06$) in samples that had been collected before brushing compared with those that had been collected at least 1 h after brushing teeth. We found no difference in hDNA yield between samples that had been collected using a 60-s swish time *versus* a 30-s swish time or in samples that were processed within 5 days of collection. However, samples that were held at room temperature and processed 10 or 30 days after collection yielded statistically significantly less hDNA. In general, ability to amplify using standard PCR protocols and the presence of high molecular weight DNA was good under all of the sample collection conditions.

Using standard PCR protocols, 100% of the 206 samples amplified fragments of 268-bp and 536-bp length, whereas in 94% of the samples, the 989-bp fragment was successfully amplified. The cause for PCR failure in these samples is not known; however, these PCR success rates are similar to those that have been reported previously (2–6, 8, 9, 12). All of the samples had sufficient amounts of hDNA (5.1–52.3 μg hDNA/sample), and the presence of high molecular weight DNA was observed. The nearly 1-kb length

Downloaded from cebp.aacrjournals.org on January 21, 2022. © 2001 American Association for Cancer Research.

tests the limits of standard PCR reactions, and it is possible that these samples could have been amplified with slight modifications to the PCR protocol.

Our study found little degradation of DNA and higher yields than have generally been reported for cytobrush collection even when samples were held at room temperature for up to 30 days (4, 6). Our results are consistent with Lum and LeMarchand (2) who reported that storage of unprocessed specimens at room temperature for 1 week did not affect DNA yield or ability to PCR amplify. In a similar study, Garcia-Closas *et al.* (4) found that storage at −80°C for up to 1 year did not significantly deplete the amount of hDNA in the samples.

Direct comparisons of our DNA yield to previous studies (1, 2, 6, 7, 9) are difficult, because most studies have not estimated the amount of hDNA in buccal cell samples. Garcia-Closas *et al.* (4) compared hDNA yield from cytobrushes with yield from the mouthwash method using samples from two epidemiological studies. One was a study of breast cancer, and buccal cell samples were collected from women with an average age of 55. The other samples were taken from a prospective study of male farmers with an average age of 49. Among the sample of women, the median amount of hDNA was 16.6 μg of hDNA/sample (compared with 1.6 μg of hDNA/two cytobrushes). Among the male farm workers, the median amount of hDNA was 27.5 μg of hDNA/sample (compared with 1.0 μg of hDNA/two cytobrushes). The higher hDNA yields we observed (34 μg of hDNA) may reflect differences in the study subjects. Our participants were researchers who volunteered for this pilot study and, thus, may adhere more closely to the sample collection instructions than members of a study cohort. Our participants were also younger (median age, 33). We do not have sufficient data to reliably investigate whether DNA yield varies by age, but future studies may want to address this issue.

Although several recent studies (4, 9, 10) have focused on the best way to collect buccal cells as a source of genomic DNA, some unresolved issues remain. There is general agreement that buccal cells can provide high molecular weight DNA of sufficient quality for PCR-based analyses. Whether buccal cell DNA performs well in emerging genomics technologies is unknown. In addition, variation in buccal cell yield by age, gender, and ethnicity has been suggested in these recent data and warrants further investigation.

In summary, buccal cell collection using this mouthwash protocol provides a good alternative to peripheral blood for obtaining a relatively large volume of high quality genomic DNA. Buccal cell collection may be especially suited for large, geographically dispersed cohorts or for studies conducted in areas where blood collection and storage is not safe or feasible.

References

1. Lench, N., Stanier, P., and Williamson, R. Simple non-invasive method to obtain DNA for gene analysis. Lancet, *1:* 1356–1358, 1988.

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

3. Harty, L. C., Garcia-Closas, M., Rothman, N., Reid, Y. A., Tucker, M. A., and Hartge, P. Collection of buccal cell DNA using treated cards. Cancer Epidemiol. Biomark. Prev., *9:* 501–506, 2000.

4. Garcia-Closas, M., Egan, K. M., Abruzzo, J., Newcomb, P. A., Titus-Ernstoff, L., Franklin, T., Bender, P. K., Beck, J. C., LeMarchand, L., Lum, A., Alavanja, M., Hayes, R. B., Rutter, J., Buetow, K., Brinton, L. A., and Rothman, N. Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. Cancer Epidemiol. Biomark. Prev., *10:* 687–696, 2001.

5. Harty, L. C., Shields, P. G., Winn, D. M., Caporaso, N. E., and Hayes, R. B. Self-collection of oral epithelial cell DNA under instruction from epidemiologic interviewers. Am. J. Epidemiol., *151:* 199–205, 2000.

6. Walker, A. H., Najarian, D., White, D. L., Jaffe, J. M., Kanetsky, P. A., and Rebbeck, T. R. Collection of genomic DNA by buccal swabs for polymerase chain reaction-based biomarker assays. Environ. Health Perspect., *107:* 517–520, 1999.

7. Tobal, K., Layton, D. M., and Mufti, G. J. Non-invasive isolation of constitutional DNA for genetic analysis. Lancet, *2:* 1281–1282, 1989.

8. Meulenbelt, I., Droog, S., Trommelen, G. J. M., Boomsma, D. I., and Slagboom, P. E. High-yield noninvasive human genomic DNA isolation method for genetic studies in geographically dispersed families and populations. Am. J. Hum. Genet., *57:* 1252–1254, 1995.

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

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

11. Greer, C. E., Wheeler, C. M., and Manos, M. M. Sample preparation and PCR amplification from paraffin-embedded tissues. PCR Methods Appl., *3* (Suppl.)*:* 113s–122s, 1994.

12. Freeman, B., Powell, J., Ball, D., Hill, L., Craig, I., and Plomin, R. DNA by mail: an inexpensive and noninvasive method for collecting DNA samples from widely dispersed populations. Behav. Genet., *27:* 251–257, 1997.

Downloaded from cebp.aacrjournals.org on January 21, 2022. © 2001 American Association for Cancer Research.

Cancer Epidemiology, Biomarkers & Prevention

AACR American Association for Cancer Research

Determinants of DNA Yield and Quality from Buccal Cell Samples Collected with Mouthwash

Heather Spencer Feigelson, Carmen Rodriguez, Andrea S. Robertson, et al.

Cancer Epidemiol Biomarkers Prev 2001;10:1005-1008.

Updated version Access the most recent version of this article at:
http://cebp.aacrjournals.org/content/10/9/1005

Cited articles This article cites 12 articles, 4 of which you can access for free at:
http://cebp.aacrjournals.org/content/10/9/1005.full#ref-list-1

Citing articles This article has been cited by 13 HighWire-hosted articles. Access the articles at:
http://cebp.aacrjournals.org/content/10/9/1005.full#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
http://cebp.aacrjournals.org/content/10/9/1005.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC)
Rightslink site.

Downloaded from cebp.aacrjournals.org on January 21, 2022. © 2001 American Association for Cancer Research.