

Short Communication

Buccal DNA Collection: Comparison of Buccal Swabs with FTA Cards

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Abstract

Collection and analysis of DNA, most commonly from blood or buccal cells, is becoming more common in epidemiologic studies. Buccal samples, which are painless to take and relatively easily collected, are often the preferred source. There are several buccal cell collection methods: swabs, brushes, mouthwash, and treated cards, such as FTA or IsoCode cards. Few studies have systematically compared methods of buccal cell collection with respect to DNA yield and amplification success under similar conditions. We compared buccal DNA collection and amplification using buccal swabs and FTA cards in 122 control subjects from our Australian case-control study of childhood acute lymphoblastic leukaemia. Buccal DNA was quantified using a real-time PCR for β -actin and genotyped at the loci of three polymorphisms (*MTHFR 677C>T*, *ACE*

I/D, and *XPD 1012G>A*). PCR was successful with DNA from buccal swabs for 62% to 89% of subjects and from FTA cards for 83% to 100% of subjects, depending on the locus. The matched pair odds ratios (95% confidence interval) comparing success of FTA cards with buccal swabs are as follows: *MTHFR 677C>T* using PCR-RFLP, 12.5 (11.6-13.5) and using real-time PCR, 130.0 (113.1-152.8); *ACE I/D* using PCR-amplified fragment length polymorphism, 3.36 (3.2-3.5); *XPD 1012G>A* using real-time PCR, 150.0 (132.7-172.3). FTA cards are a robust DNA collection method and generally produce DNA suitable for PCR more reliably than buccal swabs. There are, however, technical challenges in handling discs punched from FTA cards that intending users should be aware of. (Cancer Epidemiol Biomarkers Prev 2006;15(4):816-9)

Introduction

A growing number of epidemiologic studies involve the collection and analysis of DNA, of which blood (usually lymphocytes) and buccal cells are the two most common sources. Blood collection is not always possible or feasible, particularly in case-control studies when requesting blood can reduce control subjects' participation and thus threaten the validity of study results. Buccal samples are, therefore, often the preferred source of DNA (1). Their collection is usually painless and is generally considered more acceptable, particularly for children. Moreover, samples can be collected at home and mailed to the laboratory.

Several methods of buccal cell collection have been described, including swabs (2-4), brushes (1, 5), mouthwash (1, 5-8), and, more recently, treated cards, such as FTA (9, 10) or IsoCode cards (11). There have been few reports comparing different methods for collecting buccal cells with respect to DNA yield and success in amplification under similar conditions. Mouthwash and cytobrush collections have been compared. Garcia-Closas et al. (5) found that mouthwash produced consistently better yields than the brushes. King

et al. (1) concluded that they were equivalent for PCR reactions requiring short or intermediate DNA fragments, but that mouthwash was far superior for reactions requiring long fragments. Cozier et al. (12) compared cheek swabs and mouthwash for buccal cell collection in the Black Women's Health Study and concluded that the mouthwash was superior in terms of success of PCR and DNA yield.

Cytobrushes and mouthwashes are generally considered unsuitable for use in children. Cytobrushes are quite abrasive. Mouthwashes require participants to expectorate and may be aspirated or swallowed. Therefore, we decided to use buccal swabs or FTA cards for our case-control study into the causes of childhood acute lymphoblastic leukaemia, in which control children and their parents provide DNA samples. Both methods require the initial collection of cellular material from inside the cheek onto a swab or applicator. In the swab method, the swab coated with cellular material is allowed to dry and then placed in a sealed plastic tube; in the FTA card method, the fresh material is transferred from the applicator onto the indicating circle on the FTA card. This circle changes color when the sample is applied; when the circular collection area (4.9 cm²) is completely filled, ~100 discs for analysis can be punched from each card. One study comparing the efficacy of these techniques in 15 subjects (betel quid chewers) has been reported (10). Given the lack of information on the relative success of these two methods, we undertook a comparison study to help us decide which method to use.

Materials and Methods

The Australian Study of Causes of Acute Lymphoblastic Leukaemia in Children is a population-based case-control

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study that began in 2003. Case families are identified through the nine pediatric oncology centers in Australia, and control families through national random digit dialing. Case families provide blood samples during routine hospital visits. Control families collect buccal samples at home and mail them to the laboratory in prepaid envelopes. The study has approval from nine hospital human research ethics committees, and parents complete DNA consent forms.

For the comparison study, 122 subjects (93 children and 29 parents) were asked to collect buccal DNA using two buccal swabs and an indicating FTA Micro card. Subjects were sent detailed instructions for each method in accordance with the manufacturers' instructions. They were asked to mail the samples back to the laboratory within 24 hours of collection.

DNA was extracted from the two buccal swabs (placed in the same tube), using the heat lysis method, as the manufacturer instructed (Epicentre Biotechnologies, Madison, WI). Therefore, we did not determine which (if either) of the swabs contained usable DNA. Single 1.2-mm punches were taken from each subject's FTA card and prepared for PCR by washing thrice with 200 μ L FTA Purification Reagent and twice with 200 μ L TE buffer, as recommended by the manufacturer (Whatman International Ltd., Maidstone, United Kingdom).

All samples were genotyped for single nucleotide polymorphisms in the genes encoding methylenetetrahydrofolate reductase (*MTHFR* 677C>T) and xeroderma pigmentosum complementation group D protein (*XPB* 1012G>A) and for an insertion/deletion (I/D) polymorphism in the gene for human angiotensin-converting enzyme (*ACE*). The *MTHFR* 677C>T locus was selected as a representative and widely studied single nucleotide polymorphism. Two different genotyping methods, conventional and real-time PCR, involving different amplification primers, conditions, detection methods, and instrumentation were used to assess protocol robustness with the different genomic templates available in this study. As a significant proportion of human genetic variation involves over a million "Alu" repetitive elements dispersed throughout the genome, a representative of this class of genetic variation was sought for this study. The *ACE* gene was selected as it manifests a 287-bp Alu element insertion polymorphism in intron 16 with an insertion allele frequency of about 50% in most European populations (13). The *XPB* 1012G>A polymorphism (14) was also chosen as a less widely investigated single nucleotide polymorphism.

Genotyping for *MTHFR* 677C>T by RFLP was undertaken following a modification of the method of Skibola et al. (15). Genomic DNA was amplified by PCR using as primers: *MTHFR* 677C>T forward, 5'-TGAAGGAGAAGGTG-TCTGCGGA-3' and *MTHFR* 677C>T reverse, 5'-AGGAC-GGTGCGGTGAGAGTG-3' followed by digestion of the 198-bp product with *Hinf*I. Digestion products were separated by 12% PAGE and visualized by staining with ethidium

bromide. Genotyping for the 287-bp I/D polymorphism in intron 16 of the *ACE* gene was done using PCR to detect the amplified fragment length polymorphism according to a modified method of Rigat et al. (13). Specifically, the primer annealing temperature was raised to 63°C to prevent preferential amplification of the smaller allele in heterozygous individuals (16); 12% PAGE electrophoresis was used to distinguish the two fragment sizes of 478 bp (insertion allele) and 191 bp (deletion allele). Genotyping for *MTHFR* 677C>T and *XPB* 1012G>A (14) was done by real-time PCR using custom Taqman assays (Perkin-Elmer Applied Biosystems, Foster City, CA). The primer sequences used for *MTHFR* 677C>T were forward, 5'-GCACTTGAAGGAGAAGGTGTCT-3' and reverse, 5'-CTCAAAGAAAAGCTGCGTGATG-3'. The reporter probes were GCGGGAGCCGATTTCAT-VIC and GCGGGAGTTCGATTTCAT-FAM. For the *XPB* 1012G>A single nucleotide polymorphism, the primer sequences were forward, 5'-CCCACCTGGCCAACCC-3' and reverse, 5'-CTGCGAGGAGACGCTATCAG-3'. The reporter probes were CTGCCCGACGCCGT-VIC and CTGCCCAACGAAGT-FAM. Each PCR mixture contained 2 μ L of DNA (or one FTA punch), 0.25 μ L 40 \times Assay Mix, and 5 μ L 2 \times Taqman Universal PCR Master Mix made up to a final volume of 10 μ L with sterilized MilliQ water. PCR was done using the ABI PRISM 7900HT sequence detection system (Perkin-Elmer Applied Biosystems) at the following thermal cycling conditions: 50°C for 2 minutes, 95°C for 10 minutes, 92°C for 15 seconds, and 60°C for 1 minute, for 40 cycles.

If a sample was successfully genotyped after up to three attempts, it was classified as "pass"; if not, it was classified as "fail." Laboratory staff making this classification were blind in each case to the classification of the other sample for each subject. The matched pair odds ratios and 95% confidence intervals were calculated to compare genotyping success of DNA samples collected from the same subjects using the two different methods. A value of 0.1 was used in cells with zero values in the calculation of odds ratios and confidence intervals to avoid an indeterminate result.

All buccal swab aliquots were subjected to quantitative real-time PCR for β -actin using the following primers and probes: forward, 5'-TCACCCACACTGTGCCCATCTACGA-3'; reverse, 5'-CAGCGGAACCGCTCATTGCCAATGG-3'; and 5'-VIC-ATGCCCTCCCCATGCCATCCTGCGT-TAMRA-3'. Real-time PCR was done with a Taqman ABI Prism 7000 Sequence Detection System (Perkin-Elmer Applied Biosystems) using placental DNA as a standard.

Results and Discussion

All 122 subjects returned two buccal swabs and an FTA card, with a median time of 4 days (interquartile range of 4 days)

Table 1. Success of genotyping from FTA card and buccal swabs at three loci in 122 subjects

| Locus | Buccal swabs | FTA cards, <i>n</i> (overall %) | | |
|-------------------------------------|--------------|---------------------------------|-----------|------------|
| | | Pass | Fail | Total |
| <i>MTHFR</i> 677C>T (RFLP) | Pass | 95 (77.9) | 2 (1.6) | 97 (79.5) |
| | Fail | 25 (20.5) | 0 (0) | 25 (20.5) |
| | Total | 120 (98.4) | 2 (1.6) | 122 (100) |
| <i>MTHFR</i> 677C>T (real-time PCR) | Pass | 109 (89.3) | 0 (0) | 109 (89.3) |
| | Fail | 13 (10.6) | 0 (0) | 13 (10.6) |
| | Total | 122 (100) | 0 (0) | 122 (100) |
| <i>ACE</i> I/D | Pass | 65 (53.3) | 11 (9.0) | 76 (62.3) |
| | Fail | 37 (30.3) | 9 (7.4) | 46 (37.7) |
| | Total | 102 (83.6) | 20 (16.4) | 122 (100) |
| <i>XPB</i> 1012G>A | Pass | 107 (87.7) | 0 (0) | 107 (87.7) |
| | Fail | 15 (12.3) | 0 (0) | 15 (12.3) |
| | Total | 122 (100) | 0 (0) | 122 (100) |

Table 2. Consistency in genotyping in FTA card and buccal swabs and median DNA concentration in buccal swabs

| No. loci genotyped successfully | <i>n</i> (%) | | β-Actin concentration in buccal swab (ng/μL) | | |
|---------------------------------|--------------|-------------|--|-----------------|-----------------|
| | FTA card | Buccal swab | Median | 25th percentile | 75th percentile |
| 4 | 101 (82.8) | 59 (48.4) | 5.60 | 2.89 | 11.10 |
| 3 | 20 (16.4) | 40 (32.8) | 5.80 | 1.84 | 14.19 |
| 2 | 1 (0.8) | 11 (9.0) | 0 | 0 | 0.78 |
| 0 or 1 | 0 (0) | 12 (9.8) | 4.69 | 0.83 | 10.33 |

between the samples being collected and their receipt at the laboratory. For the majority of FTA cards, over half the indicating circle was covered with sample, allowing more than fifty 1.2-mm discs to be punched.

Genotyping success was greater with FTA card than swab samples for each locus and amplification method (Table 1). The matched pair odds ratios (95% confidence intervals) for the four polymorphisms [*MTHFR* 677C>T (RFLP), *MTHFR* 677C>T (real-time PCR), *ACE* I/D, and *XPD* 1012G>A] were 12.5 (11.6-13.5), 130.0 (113.1-152.8), 3.4 (3.2-3.5), and 150.0 (132.7-172.3), respectively; 101 (82.8%) of the FTA cards and 59 (48.4%) of the swab samples were successfully genotyped at all loci (Table 2).

DNA derived from both sample types had a higher success rate when genotyped for *MTHFR* 677C>T using real-time PCR than when using conventional RFLP analysis (89% versus 80% for swabs and 100% versus 98% for FTA cards; Table 1), possibly reflecting the more sensitive allelic discrimination capacity of real-time PCR.

β-Actin concentrations for the swab samples ranged from indeterminate (<0.01 ng/μL) to 34.35 ng/μL, with a median of 4.97 ng/μL and an interquartile range of 1.53 to 11.38 ng/μL. The median concentration of β-actin varied little among swab samples that amplified successfully at four, three, one, or no locus; more than half the swab samples that amplified at only two loci had an indeterminate β-actin result; thus, the median concentration in that group was classified as "zero" (Table 2). Thus, there was no apparent association between β-actin concentration of the swab material and the number of loci successfully amplified. Furthermore, swab β-actin concentration was not related to the success of amplification at any individual locus (data not shown).

Double-stranded DNA bound to FTA discs cannot be released for quantification without restriction endonuclease digestion (Whatman Application Note). Thus, the discs used for genotyping in this study could not be directly assessed for DNA content in the β-actin assay, as they could be used in only one PCR amplification. Moreover, repeated sampling from the same FTA card yielded different DNA content in β-actin assays, suggesting that the distribution of DNA template is not uniform on the card, and that the DNA content of a disc used for genotyping cannot be reliably estimated by assessing a different disc for quantitative PCR of β-actin.

Genotyping success from buccal swabs has been shown to vary significantly across different studies in accordance with the method of collection, postage time, and method of DNA extraction. Success rates for posted buccal cytobrush or swab samples for loci of ~500 bp have varied from 20.0% to 97.5% (2, 5), with our success rate of 62.3% for the 191-bp/487-bp *ACE* I/D polymorphism lying between these extremes. Some of this variability can be attributed to the differing DNA extraction methods, which can influence DNA yield and genotyping success. Garcia-Closas et al. (5) compared phenol-chloroform, a commercial blood extraction kit, and NaOH extraction in obtaining buccal DNA from cytobrushes and found that the PCR success rate for a 536-bp fragment varied from 97.5% to 92.5% to 20.0%, respectively, with human DNA

yields varying from 1.0 to 0.4 to 1.3 μg, respectively. However, the percentage of total DNA that was human obtained from the cytobrushes was small (7.3%, 4.5%, and 1.0%, respectively), and high molecular weight DNA (>23 kb) varied concomitantly (55%, 2.5%, and 0.0%, respectively), suggesting bacterial contamination. As several buccal swabs in our study were visibly affected by bacterial or fungal growth, the impregnation of FTA cards with nuclease inhibitors, which inhibit DNA degradation, and with antimicrobial agents, which inhibit bacterial and fungal growth, may be important to their greater success.

The FTA cards also protect the DNA from UV radiation damage and contain reagents that stabilize and immobilize the DNA directly in the card matrix. This sequestering of the DNA allows any PCR inhibitors to be removed during the washing steps done before PCR, resulting in better amplification of buccal DNA from FTA cards than that extracted from swabs (10). The FTA cards have other advantages. Used cards can be archived at room temperature for >10 years before being analyzed compared with 5 days for swabs. This also means that delays in postage or transport are less likely to result in degraded DNA. The total cost of preparation of PCR-ready DNA in our hands was about 28% less using FTA cards (US\$4.46) than two swabs (US\$6.19).

We had some difficulty processing the FTA cards in the laboratory. The 1.2-mm punches are taken from the circle containing the DNA and placed in a 0.5-mL tube. The punched discs are washed several times and then dried at room temperature. PCR reactions are typically undertaken in 0.2-mL tubes, which require the transfer of the dried discs to the new tube. Given the size of the discs, this process is difficult and increases the probability of laboratory error or contamination. Ideally, all PCR reactions would be done in the 0.5-mL tube, although many laboratories may not possess thermal cyclers equipped to handle tubes of this size.

There are some other potential disadvantages of FTA cards, in that they generally produce a smaller sample than the swabs, and may require a higher PCR volume. However, we have found that these issues are countered by the fact that the number of assays done on a single FTA card can be extended via whole genome amplification of punches, or by washing and reusing the punches, and that amplification is successful after fewer attempts.⁷

The results of our study are likely to apply to other groups of subjects. The control subjects who participated in this substudy comprised all subjects recruited from the general population at that time.

In conclusion, FTA cards are a robust DNA collection method that produces usable DNA for PCR more reliably than do buccal swabs. The collection of material for the FTA cards is simple and pleasant compared with mouthwash or cytobrushes. The challenges associated with handling the small discs are minor compared with the advantages of FTA cards.

⁷ L.J. Ashton, unpublished observations.

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References

- King IB, Satia-Abouta J, Thornquist MD, et al. Buccal cell DNA yield, quality, and collection costs: comparison of methods for large-scale studies. *Cancer Epidemiol Biomarkers Prev* 2002;11:1130–3.
- Walker AH, Najarian D, White DL, et al. Collection of genomic DNA by buccal swabs for polymerase chain reaction-based biomarker assays. *Environ Health Perspect* 1999;107:517–20.
- Kozlowski LT, Vogler GR, Vandenberg DJ, et al. Using a telephone survey to acquire genetic and behavioral data related to cigarette smoking in “made-anonymous” and “registry” samples. *Am J Epidemiol* 2002;156:68–77.
- van Rooij IA, Wegerif MJ, Roelofs HM, et al. Smoking, genetic polymorphisms in biotransformation enzymes, and nonsyndromic oral clefting: a gene-environment interaction. *Epidemiology* 2001;12:502–7.
- Garcia-Closas M, Egan KM, Abruzzo J, et al. Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. *Cancer Epidemiol Biomarkers Prev* 2001;10:687–96.
- Heath EM, Morken NW, Campbell KA, et al. Use of buccal cells collected in mouthwash as a source of DNA for clinical testing. *Arch Pathol Lab Med* 2001;125:127–33.
- Feigelson HS, Rodriguez C, Robertson AS, et al. Determinants of DNA yield and quality from buccal cell samples collected with mouthwash. *Cancer Epidemiol Biomarkers Prev* 2001;10:1005–8.
- Le Marchand L, Lum-Jones A, Saltzman B, et al. Feasibility of collecting buccal cell DNA by mail in a cohort study. *Cancer Epidemiol Biomarkers Prev* 2001;10:701–3.
- Gutierrez-Corcherro F, Arruga M, Sanz L, et al. Using FTA cards to store avian blood samples for genetic studies. Their application in sex determination. *Mol Ecol Notes* 2002;2:75–7.
- Salvador JM, De Ungria MC. Isolation of DNA from saliva of betel quid chewers using treated cards. *J Forensic Sci* 2003;48:794–7.
- Harty LC, Garcia-Closas M, Rothman N, et al. Collection of buccal cell DNA using treated cards. *Cancer Epidemiol Biomarkers Prev* 2000;9:501–6.
- Cozier Y, Palmer J, Rosenberg L. Comparison of methods for collection of DNA samples by mail in the Black Women’s Health Study. *Ann Epidemiol* 2004;14:117–22.
- Rigat B, Hubert C, Corvol P, Soubrier F. PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1). *Nucleic Acids Res* 1992;20:1433.
- Hemminki K, Xu G, Angelini S, et al. XPD exon 10 and 23 polymorphisms and DNA repair in human skin *in situ*. *Carcinogenesis* 2001;22:1185–8.
- Skibola CF, Smith MT, Kane E, et al. Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. *Proc Natl Acad Sci U S A* 1999;96:12810–5.
- van Bockxmeer FM, Mamotte CD, Gibbons FA, Burke V, Taylor RR. Angiotensin-converting enzyme and apolipoprotein E genotypes and restenosis after coronary angioplasty. *Circulation* 1995;92:2066–71.