ORAcollect®•DNA

Comparison of DNA from samples collected using ORAcollect®•DNA (OCR-100) vs. buccal swabs

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Introduction

DNA based genetic testing is becoming increasingly integrated into standard medical practices. This has led to a growing need for a source of DNA that is not only reliable, easy to use and of high quality, but also cost effective. Historically, blood or buccal swab samples have been used as the DNA source; however these sample types are accompanied by unique challenges. In the case of blood, the invasive nature of the sample collection coupled with special handling requirements (e.g., venipuncture, immediate processing, and cold storage/transport), makes it an expensive choice in terms of reduced donor compliance and increased logistical costs. Conversely, for buccal swabs, the perceived low cost sample collection is off-set by the poor quality of the DNA obtained, often resulting in repeat sampling, which reduces convenience, impacts patient care and increases cost.

ORAcollect•DNA (OCR-100) has been optimized to overcome these issues by providing an easy-to-use, high quality sample which provides the following benefits:

- Minimally invasive sample collection which can be performed in the absence of medical supervision, allowing for increased donor compliance and convenience
- Liquid based samples with a standardized tube format, allowing for improved lab workflow efficiency and automated DNA extraction
- DNA in ORAcollect•DNA samples is stabilized at room temperature when collected, removing the requirement for costly refrigeration post-collection and the potential for sample degradation
- ORAcollect•DNA chemistry is bacteriostatic, inhibiting the growth of bacteria from the time of sample collection to processing.

All of these factors make ORAcollect•DNA oral samples an attractive source of DNA for use in genomic technologies such as PCR. This technical bulletin describes the quality, yield and performance of oral sample DNA collected using the ORAcollect•DNA kit as compared to buccal swabs.

Materials and methods

DNA metrics studies

Oral samples were obtained from 44 donors using either the ORAcollect•DNA kit or a standard sterile cotton swab (AMG Medical Inc., Montreal, QC). Collection of ORAcollect•DNA samples was carried out according to the ORAcollect•DNA protocol and purification of DNA was carried out using the prepIT®•L2P protocol[1] for OCR-100, and scaled down to 250 µL. Buccal samples were collected using a sterile cotton swab to swab the inside of both cheeks for 20 seconds each. DNA was purified using the Qiagen® Blood Mini Spin Kit (Qiagen Sciences, Maryland, USA) and the Buccal Spin Protocol. For the ORAcollect•DNA samples, the starting sample to elution volume was 250:50 (a starting chemistry/oral sample volume of 250 µL, with an elution volume of 50 µL. For the cotton swab samples, the entire swab was processed, as directed by the protocol. DNA yield was determined by fluorescence using the Pico® Green I dye (Invitrogen) and a TECAN Infinite® M200 microplate reader. Briefly, purified DNA was first diluted 1:50 in 1X TE (10mM Tris-HCl, 1mM EDTA, pH 7.5). A Lambda DNA standard with concentrations ranging from 10 to 0.156 ng/µL was prepared by serial dilution with 1X TE. 5 µL of diluted DNA (both standards and unknown) was mixed with 95 µL 1X PicoGreen® in a 96-well black microplate and incubated at room

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temperature for 5 minutes. Using the TECAN Infinite® M200 microplate reader, samples were excited at 485 nm, and fluorescent emission was read at 535 nm. DNA purity was assessed by UV absorbance at 260 nm, 280 nm and 320 nm. A fraction (100 ng each) of the purified DNA was run on a 0.8% agarose gel in order to assess DNA quality.

Sample stability study

Oral samples were obtained from 18 donors using either the ORAcollect•DNA kit or a standard sterile cotton swab. Two cotton swabs were collected from each donor to allow later processing. DNA was purified as described above. Bacterial DNA content was determined according to protocol PD-PR-065[2]. The samples were stored at room temperature, and aliquots (ORAcollect•DNA) or whole sample (buccal swab), were processed at 1 and two months.

DNA performance in downstream applications

Three samples each (ORAcollect•DNA or buccal swab) were randomly selected from the DNA Metrics Study for analysis of performance using long-range PCR. A 3.6 kb fragment of the human CYP2D6 (Cytochrome P450 2D6) gene was amplified using sequence specific primers. The PCR products were run on a 0.8% agarose gel in order to assess DNA performance.

Results

ORAcollect•DNA provides high quality, high quantity DNA compared to buccal swabs

The DNA yields obtained with ORAcollect•DNA samples or from buccal swab samples is shown in Figure 1. The mean DNA yield is significantly higher for ORAcollect•DNA samples (4.07 µg) as compared to buccal swabs (0.87 µg), with 95% of ORAcollect•DNA samples yielding more than 1.1 µg. Median yields are 3.9 and 0.7 µg, respectively (see Table 1). The DNA collected from ORAcollect•DNA samples is also of a higher purity and integrity as compared to buccal swabs. UV absorbance analysis was performed as an initial assessment (Table 2). DNA from buccal swabs gives comparable readings to ORAcollect•DNA samples, however due to the extremely low concentration; five buccal swab samples had to be excluded due to insufficient DNA content. Analysis by visualization on a 0.8% agarose gel also showed that DNA from buccal swabs is largely degraded as compared to DNA from ORAcollect•DNA samples; a representative gel image is shown in Figure 2.

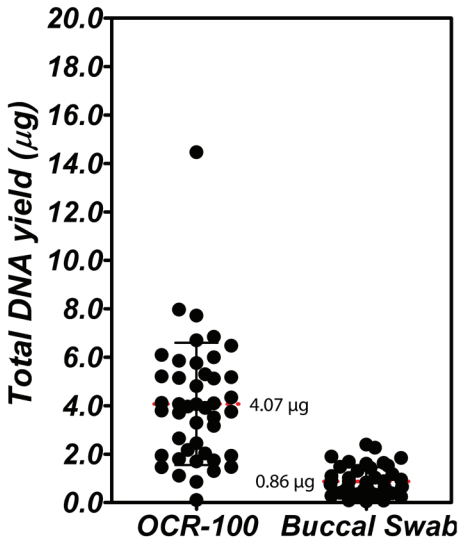

***Figure 1**: Total DNA yield obtained from oral samples.*
Scatter dot plot of the total DNA yields in ORAcollect•DNA and buccal swab samples. The dashed red lines represent the mean yields. Black lines indicate the standard deviation. N=44

	OCR-100	Buccal swab
Mean	4.07	0.86
Median	3.9	0.7
Minimum	0.11	0.05
Maximum	14.47	2.40
5th percentile	1.14	0.16

***Table 1**: Overview of total DNA yield (µg) comparison from OCR-100 and buccal swabs.*

	OCR-100	Buccal swab†
Average	1.7	1.8
Median	1.8	1.8
Minimum	1.5	1.4
Maximum	1.9	2.6
SD	0.07	0.23

***Table 2**: UV Absorbance ratio (A_{260}/A_{280}) of DNA purified from ORAcollect•DNA and buccal swabs.*

The average, median, minimum and maximum values for the measured absorbance readings are listed together with the standard deviations. N=44

† Five buccal swab samples were not included in the analysis due to insufficient amounts of DNA.

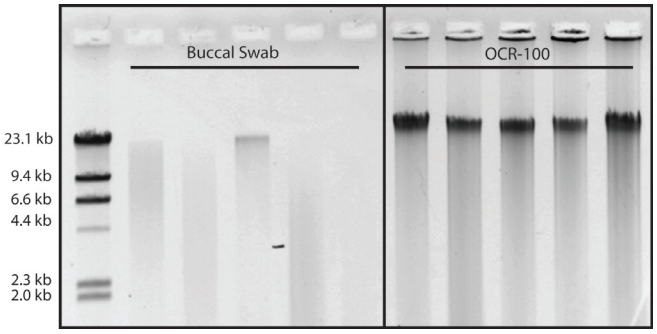

***Figure 2**: Agarose gel analysis of DNA integrity from ORAcollect•DNA and buccal swab samples.*

Agarose gel electrophoresis of DNA purified from buccal swabs (left) and ORAcollect•DNA kits (right). 100 ng of each sample was loaded. A Lambda-HindIII digest was used as a marker in lane 1 (DNA Ladder).

ORAcollect•DNA stabilizes the sample and prevents bacterial growth

The percentage of bacterial DNA in oral samples was monitored over time. In ORAcollect•DNA samples, there is no significant increase in bacterial DNA content after storage for 60 days at room temperature (Figure 3, left panel). This is due to the bacteriostatic nature of the ORAcollect•DNA chemistry. In buccal swab samples, however, a significant increase in bacterial DNA content is seen after only 30 days at room temperature (Figure 3, right panel).
An overview of bacterial DNA present in OCR-100 and buccal swab samples is presented in Table 3.

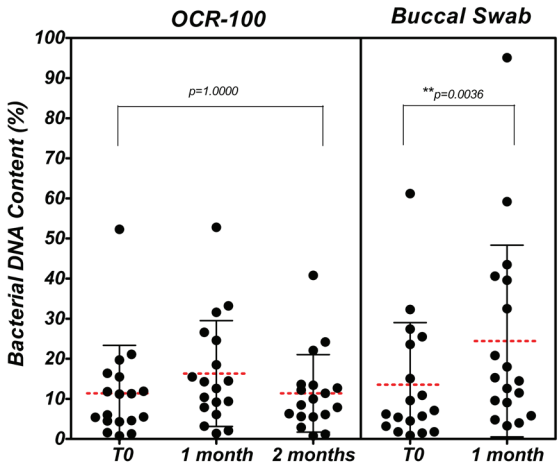

***Figure 3**: Bacterial DNA content in oral samples.*

Scatter dot plot of the percentage of bacterial DNA present in ORAcollect•DNA and buccal swab samples. The samples were analysed by PCR analysis at 1 and 2 months (ORAcollect•DNA only). The dashed red lines represent the mean bacterial DNA content. Black lines indicate standard deviation. N=18

	OCR-100	Buccal swab
Mean T_0	11%	14%
Mean T= 1 month	16%	24%
Mean T- 2 months	11%	n/a‡

***Table 3**: Overview of bacterial DNA content (% DNA) comparison from OCR-100 and buccal swabs.*

‡ No data

ORAcollect•DNA provides DNA suitable for complex downstream applications

DNA collected using either ORAcollect•DNA or buccal swabs was used as a template for the long-range PCR amplification of a 3.2 kb portion of the CYP2D6 gene. A band of the expected size is clearly seen in all ORAcollect•DNA samples (Figure 4, right panel). There is no amplified product present in any of the buccal swab samples.

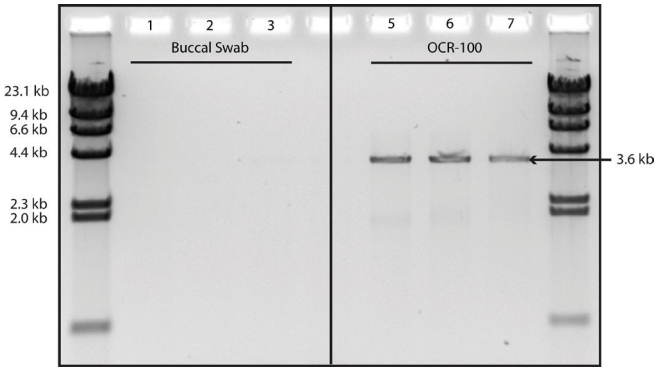

Figure 4: Agarose gel of Long-range PCR reactions.

Lanes 1-3 used 8 uL of amplified DNA extracted from buccal swabs. Lanes 5-7 used 8 uL of amplified DNA extracted from ORAcollect•DNA/saliva. Lane 4 is empty.

Conclusions

ORAcollect•DNA is a non-invasive DNA self-collection kit that can be used for obtaining high quality DNA from oral samples. ORAcollect•DNA provides DNA that is superior in yield, quality and downstream performance as compared to buccal swabs.

References

1 Laboratory protocol for manual purification of DNA from 0.5 mL of sample. DNA Genotek. PD-PR-006.
2 Bacterial DNA assay. DNA Genotek. PD-PR-065.

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