Diagnostic Assessment of ORAGENE DNA Self-collection kit

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Introduction

Genomic DNA from peripheral blood is the most commonly used source of DNA when testing for germline point mutations, small indels or large genomic rearrangements in the breast cancer genes (BRCA1 and BRCA2) and the Hereditary Non-Polyposis Colorectal Cancer genes (MLH1, MSH2, MSH6 and PMS2). In this study we have evaluated a new technology of sample collection, the Oragene DNA self-collection kit (Oragene, DNA Genotek, USA), which utilised saliva as a source of genomic DNA. The company states that the yield of DNA, DNA quality and quantity is comparable to the yield obtained from blood and can be successfully utilised in downstream applications. In saliva, DNA is found in the white blood cells and the buccal epithelial cells of the mouth. Collecting patient saliva via the Oragene technology can offer clinicians and patients a safer, non-invasive and reliable alternative to collecting blood. There are three different kits on the market, the DISC format, the TUBE and the VIAL format, our laboratory trialled the VIAL format (OG-100) (Figure 1.).

Figure 1. Oragene DNA self-collection kit formats

Aim

The aim of this study is to provide a comparable and confident alternative for patients and clinicians to the commonly used collection of peripheral blood.

Method

Ten Familial Cancer Clinic patients, previously tested for genetic mutations using DNA extracted from the patient's peripheral blood, were blindly re-tested for the same mutations using their saliva DNA. The 10 patients were sent the Oragene DNA self-collection kit, in Vial Format (OG-100), and followed the kit insert instructions on how to take their own sample, PI 001 Rev 1.0 Feb14, 2004. The samples were sent to Peter MacCallum Cancer Centre-Molecular Pathology via the mail. To evaluate the stability of the DNA collected by the Oragene collection kit, the saliva specimens were subjected to 120 hours heat treatment (65ºC) and further prolonged storage for 9 months at room temperature. Thus, the saliva DNA were tested at three saliva storage checkpoints: at time of specimen collection (Saliva RT); after 120 hours at 65ºC (Saliva 120H@65); and after 120 hours at 65ºC and stored for 9 months at Room Temperature (Saliva 120H@65+9m@RT). The saliva was processed in the PMCC laboratory and the DNA was extracted from 500µl of saliva according to the manufacturers' recommendations. The DNA concentration was measured using an optical density reader (BioPhotometer, Eppendorf) to measure the quality and yield of the samples (Table 2). Peripheral blood was collected in EDTA tubes and DNA was extracted with the QIAGEN DNA Mini Kit. Each sample was assigned a specific gene test according to the genetic counsellors' request. The tests ranged from point mutations, small insertion/deletions to large genomic rearrangement screenings in either the BRCA1 and BRCA2 genes or the HNPCC genes (Table 1). The required PCR analysis and 2% agarose gel electrophoresis was completed, followed by either direct sequencing with BDTv3.1 or Multiplex Ligation Probe Analysis (MLPA).

Results and Discussion

Blind testing revealed that both methods of obtaining DNA gave similar quality, quantity and test outcomes. The DNA extracted from saliva samples gave acceptable yields (2.5-20µg/500µl saliva), DNA concentration ranged between 49.7 - 410ng/µl, and the quality of the DNA ranged between 1.41 - 1.87 ($A_{260/280}$). DNA extracted from blood revealed a higher quality ($A_{260/280}$) (1.703, STD=0.091) compared to the DNA prepared from saliva. However, the DNA quality value $A_{260/230}$ for DNA from blood was the lowest (1.266, STD=0.462) indicating the presence of an elevated level of carbohydrates and lipids in DNA solution. The best DNA yield was obtained with DNA preparation from saliva samples subjected to 65ºC for 120 hours followed by storage at RT for 9 months (19.8µg). The gene sequencing and MLPA analysis of DNA from saliva revealed satisfactory quality chromatograms with minimal or no background (refer to Figures 2 and 3 for example results). The sequencing quality score for DNA specimens from saliva were significantly higher than the score for DNA from blood (Table 3). MLPA results were comparable between two DNA collection – extraction procedures. Both of them revealed clean and easy data for analysis and interpretation. Thus, for all ten patients the gene mutation test results obtained with saliva DNA correlated well with the mutation results obtained from blood DNA. We were able to identify correctly the mutations in all blindly provided saliva specimens with a high level of specificity and sensitivity.

Patient	Test Requested	Test Method	Analysis Results
Patient 1	BRCA2 ex11	Sequence	A2 ex11 4075delGT
Patient 2	MSH2 ex13	Sequence	H2 ex13 2131 C>T
Patient 3	BRCA2 ex18	Sequence	A2 ex18 IVS17-1 G>C
Patient 4	MSH1 ex8	Sequence	H1 ex8 672delT
Patient 5	BRCA2 ex11	Sequence	A2 ex11 5466insT
Patient 6	BRCA2 ex18	Sequence	A2 ex18 IVS18+1 G>A
Patient 7	BRCA1	MLPA	A1 Dup13
Patient 8	BRCA2 ex22	Sequence	NEGATIVE
Patient 9	BRCA1	MLPA	A1 del5
Patient 10	BRCA1	MLPA	A1 del14-20

Table 1: Mutation tests requested for each of the 10 patient samples and the corresponding results.

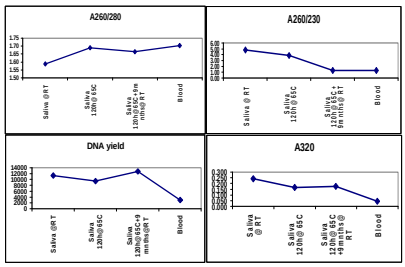

Table 2: Comparison of the quality and yield of DNA extracted at different times and temperatures

A260/280 value should be 1.7-2.0 (contamination of residual protein)
A260/230 value should be >1.1 (absence of carbohydrate, lipid and residual phenol)
A320 is background (the lower the value the better; usually between 0.1-0.01)

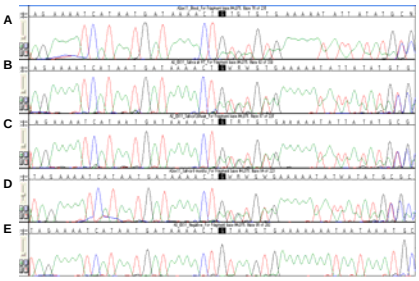

Figure 2. Comparison of sequencing results for Patient 1 BRCA2 exon 11 4075 del GT

A - Peripheral Blood Sample
B - Saliva Sample - at room temperature
C - Saliva Sample -120 hours heat treated
D - Saliva Sample - nine month old saliva stored at room temperature
E - Negative Control
Sequencing data were aligned with reference sequences: BRCA2 mRNA U43746

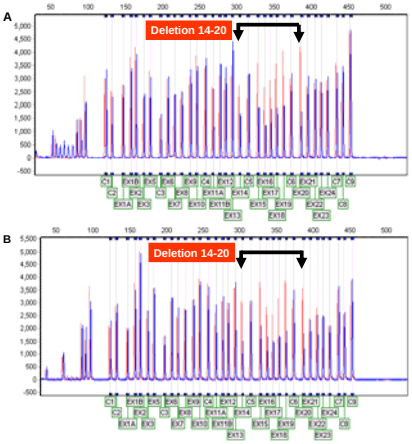

Figure 3. Comparison of MLPA results for Patient 10 BRCA1 Deletion of exons 14-20

A - Peripheral Blood Sample
B – Saliva Sample - nine month old saliva stored at room temperature

Red Peaks – Negative Control peaks for BRCA1
Blue Peaks - Saliva patient 10 – BRCA1 Deletion of exons 14-20

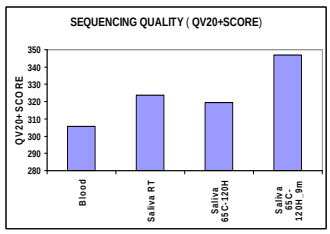

Table 3: Sequencing quality analysis based on Phred20 score

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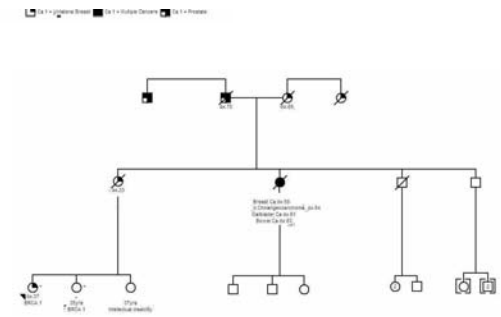

Figure 4. Pedigree of the patient with intellectual disability

A 37 year old patient with an intellectual disability and a 50% risk of a BRCA1 mutation was sent the Oragene DNA self-collection kit under her guardians (her two sisters) decision to proceed with testing on grounds of future management plans. The patient had a fear of medical procedures, particularly of needles, and had incidents of past traumatic experiences with blood collection. This non-invasive alternative was offered to the patient, which was easy to use, stress free and involved active participation of the patient in a less intimidating circumstance. Through saliva collection a viable sample was obtained and a reliable result was concluded.

Conclusions

With both methods giving concurrent outcomes it suggests that the laboratory could offer clinicians and patients an alternative way of collecting genetic material and still have the same confidence in the results. This non-invasive saliva kit could benefit patients in remote or rural areas as the kit makes it possible for the patient to take their own sample and send it by mail, without fear of heat degradation to the sample. In addition, the Oragene DNA self-collection kit would allow ease of use for patients with disabilities or in cases where blood samples cannot easily be collected from the patient. From our analysis to date it suggests that the saliva can be stored for long periods of time in the kit with limited loss of DNA quality and with comparable results to that of testing fresh samples. Using saliva instead of blood provides a safer environment for sample collection, to the clinician and the patient, eliminating the risk of blood born infections. From our current assessment of this technology it suggests that the Oragene DNA self-collection kit would be a reliable and safe alternative to peripheral blood collection.

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References

1. Rylander-Rudqvist,T., Hakansson,N., Tybring,G., & Wolk,A. Quality and quantity of saliva DNA obtained from the self-administrated oragene method--a pilot study on the cohort of Swedish men. *Cancer Epidemiol. Biomarkers Prev.* 15, 1743-1745 (2006).
2. Rogers,N.L., Cole,S., Lan,H.-C., Crossa,A., & Demerath,E.W. New saliva DNA collection method compared to buccal cell collection techniques for epidemiological studies. *American Journal of Human Biology* 19, 319-326 (2007)
3. Jin,Y. *et al.* NALP1 in vitiligo-associated multiple autoimmune disease. *N. Engl. J. Med.* 356, 1216-1225 (2007).