Saliva specimens collected with Oragene ONE are a reliable alternative to blood and buccal swabs for large scale DNA extraction and HLA typing of recruits for Hematopoietic stem cell donor registries.

McWhinnie *et al.* 24th EFI conference, Florence 2010 ; Poster presentation.
The Anthony Nolan Laboratories and UCL Cancer Institute, Royal Free Campus, London, UK.

Introduction

The use of saliva as an alternative to buccal swabs and mouthwashes for DNA isolation and genetic analyses (Ref.1) has led to the development of enhanced self collection systems such as Oragene from DNA Genotek (Ottawa, Canada) whose kits incorporate reagents for the preservation and pre-treatment of cells present in saliva prior to laboratory processing. The nature of the specimen i.e. a plastic tube with stabilised cells in liquid suspension facilitates sample storage and, save for a short initial incubation, DNA extraction by simple manual procedures or loading onto an automated workstation. The successful use of Oragene saliva as a means of self collection has been reported (Ref.2) and now DNA extracted from Oragene saliva specimens are being increasingly used for clinical and research applications such as the genotyping of Crohn's Disease patients (Ref.3) and the successful HLA typing of AML patients with low WBC counts (Ref.4). As part of a review of Anthony Nolan Trust donor recruitment we investigated the move from blood to self collection specimens for molecular HLA typing of volunteers wishing to join our registry. Scientific publications and personal recommendations from colleagues at the North West Thames Regional Genetics service led to a belief that an Oragene system would be amenable to high throughput automated DNA extraction, with isolate yield and quality acceptable for our current molecular typing requirements and the flexibility to support future developments.

Saliva collection system

The saliva kits used during our testing (Fig.1) consist of a collection tube with a 0.5ml specimen "fill line", a two piece hinged unit comprising a sampling cup and sealed pod (containing preservation reagent), and a "clam shell" container for pre and post sampling transit. Saliva deposited in the cup drains into the tube to the fill level. The pod is closed over and the resultant piercing of a film seal allows 1.7ml of collection reagent to combine with the saliva maintaining the viability of the specimen. The cup/pod unit is removed and replaced with a cap (not shown) before placing the sealed tube in the "clam shell" for return to the laboratory. Specimens are still viable after 2 month storage at room temperature and can be frozen long term.

Figure 1. Oragene ONE collection Kit. *Key: A-specimen tube, B-sampling cup, C-reagent pod and D- container.*

Laboratory Methods

For the three stages of our investigations DNA extractions were performed with single or 96 block silica membrane columns (Qiagen). DNA solution concentrations were not normalised prior to PCR for HLA typing with techniques routinely employed in our laboratory namely LABType SSO (One Lambda), Dynal Autoreli SSO (Invitrogen), SSP (Olerup) and "in house" SBT.

Stage 1

Initially we looked at DNA from Oragene saliva in comparison to buccal scrapes with nylon bristle cytology brushes (Fisher) and Omniswabs (Whatman). Five staff members provided specimens via each means of self collection followed by manual extraction with QIAamp minikit from 400µl of saliva and buccal cell/PBS suspensions. The concentration of extracts was measured and neat DNA was ran on agarose gels before PCR amplification of 5´UTR to 3´UTR HLA-A. All DNA were also HLA-A, B, C, DRB1/3/4/5 and DQB1 typed (34 tests per extraction procedure) with LABType SSO.

Results/Discussion

The mean DNA yields for saliva, brush and swab were 4.8µg (24ng/µl), 6.6µg (33ng/µl) and 2.0µg (10ng/µl) respectively. The electrophoresis of 100ng DNA from each collection method exhibited a large intact fragment(>10kb) with Oragene and fragmented smears from the buccal isolates (Fig 2). Successful "full length" HLA-A PCR product (>3kb) was achieved with 25ng DNA from all saliva but was less reliable with buccal extracts (Fig 3). HLA typing results at the loci tested were in agreement across the three extraction procedures with saliva and brushes failing 1/34 tests and swabs 3/34. All DNA successfully amplified fragments (<1kb) required for SSO typing but "full length" PCR suggests that the level of large intact DNA present in saliva preparations is more accommodating across a wider range of typing protocols.

Figure 2. 100ng of genomic DNA from self collection procedures after agarose gel electrophoresis.
Key: The 5 staff samples are named A to E; wells 2 - 6 Oragene saliva, wells 8 - 12 cytology brushes and wells 14 - 18 swabs. Wells 1,7,13 and 19 are loaded with Hyperladder I (Bioline) maximum fragment size 10kb.

Figure 3. HLA-A full length PCR (3.2kb) of 25ng DNA from self collection procedures.
Key: The 5 staff samples are named A to E; wells 2 - 6 Oragene saliva, wells 8 - 12 cytology brushes and wells 14 - 18 swabs. wells 1,7,13 and 19 are loaded with Hyperladder I (Bioline).

Stage 2

At a recruitment clinic 43 new donors provided both a blood sample and an Oragene ONE specimen. The bloods were processed as routine i.e. semi automated DNA extraction from 200µl sample with QIAamp 96 kits on a ROSYS workstation before HLA-A,B,C and DRB1 typing (172 tests) with LABType SSO. Any LABType fails are repeated with Dynal Autoreli SSO and donor HLA is entered on the registry database. In the case of the Oragene samples an MDx fully automated extraction robot isolated DNA from 200µl saliva using the same Qiagen kit with a programmed blood protocol prior to LABType SSO. DNA yields and typing results obtained from both collection methods were compared. In addition 16 selected saliva DNA were referred for Class I SBT testing.

The mean DNA yield from saliva was 3.6µg (18ng/µl) compared to 8.4µg (42ng/µl) from the blood specimens. PCR amplification for LABType of the 43 blood and saliva isolates was successful for all 4 loci (Fig.4). Resultant HLA typing of the saliva DNA was in agreement with findings from the routine blood processing with 13, 21,12 and 12 allele groups, of HLA-A, B, C and DRB1 respectively, observed in the 43 donors. There were 0% SSO repeats required from saliva DNA from the 172 tests performed compared to a routine rate of 1.8%. Class I PCR of Intron 1 to 3´UTR of HLA-A, B and C for SBT was successful with saliva DNA (Fig.5) and exon 2/3/4 sequence typing in agreement with SSO results.

Although DNA yield from saliva was on average lower than that from blood it was still sufficient to allow SSO typing with the reliability of blood samples. Saliva DNA was also suitable for our "in house" SBT.

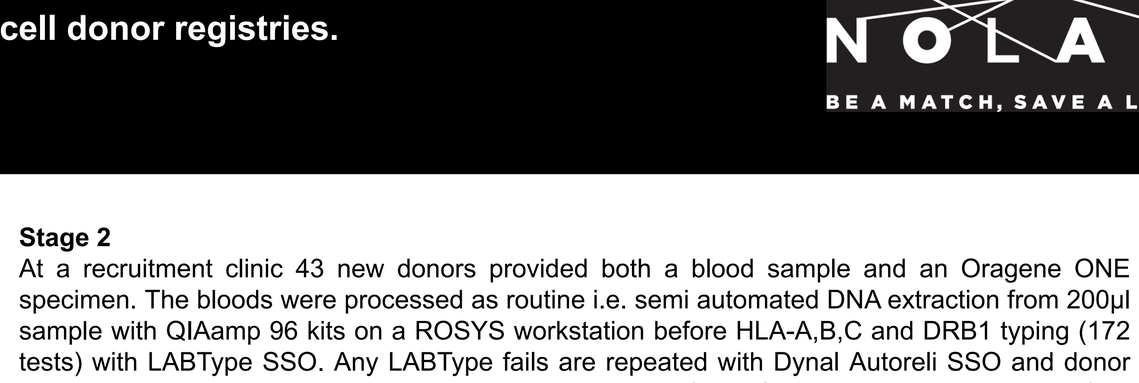

Figure 4. Examples of products from amplification of saliva DNA with Class I and II LABType PCR protocols.
Key: Upper Row - HLA-B PCR; wells 1 and 18 Hyperladder IV (Bioline), well 2 +ve control DNA, well 3 Blank reaction and wells 4 - 17 DNA from 14 new registry donors.
Lower Row - HLA-DRB1 PCR, layout as above.

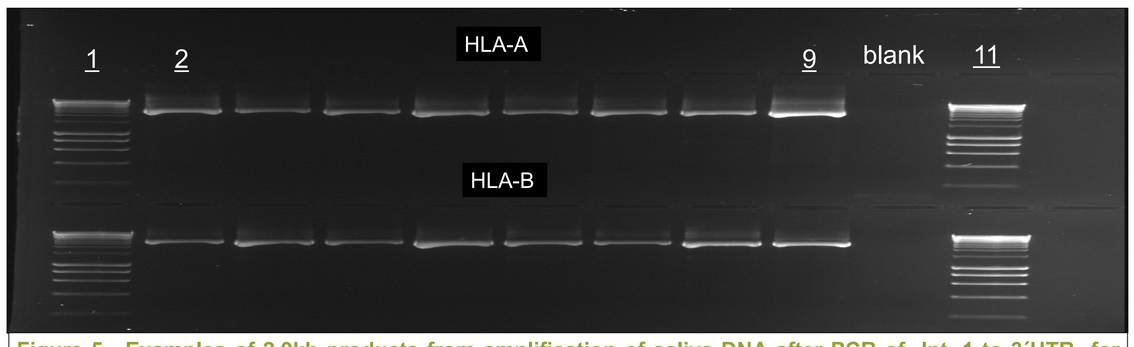

Figure 5. Examples of 2.9kb products from amplification of saliva DNA after PCR of Int. 1 to 3´UTR for Class I SBT.
Key: Upper Row - HLA-A PCR; wells 1 and 11 Hyperladder I (Bioline), wells 2 - 9 products from 8 new registry donors and well 10 blank reaction.
Lower Row - HLA-B PCR, layout as above.

Figure 6. DRB1*07 SSP result after PCR of saliva DNA from a DRB1*07:01 donor.
Key: wells 1 and 16 Hyperladder IV (Bioline) , wells 2 - 15 are the 14 SSP PCR exhibiting the lower sized specific positive products in wells 2 and 3 with the upper positive control product visible in all wells.

Stage 3

Oragene ONE was our choice for specimen self collection as part of a trust wide pilot project looking at recruitment procedures. It has been used "large scale" for clinic and postal kit samples and to date over 8000 new donors have been HLA typed from Oragene ONE saliva.

Results/Discussion:

On investigating Oragene saliva DNA from the first 564 donors a mean DNA yield of 3.0µg (15ng/ul) was observed with an HLA-A, B, C and DRB1 LABType (2256 tests) fail rate of only 1.1%. During this phase saliva DNA has been successfully used for Dynal Autoreli SSO as well as group specific SSP analysis (Fig.6).

Conclusions

Oragene ONE has allowed a problem free transition from blood collections to saliva as a source material for HLA typing during donor recruitment to the Anthony Nolan Trust registry. It offers the self collection benefits of buccal swabs in combination with the advantageous liquid handling and DNA extract qualities possible with blood. In addition the extended sample viability alleviates the concerns regarding transit and laboratory delays we experience with blood. Oragene saliva DNA has been used successfully for SSO, SSP and SBT HLA typing with results comparable to blood sample processing. At present Oragene ONE accounts for 68% of new donor specimens, with a complete move to this mode of collection planned for July 2010.

References
(1) Evaluation of saliva as a source of human DNA for population and association studies. *D. Quinque et al. Analytical Biochemistry : 353 (2006): 272-277*
(2) Quality and Quantity of Saliva DNA obtained from the Self-administered Oragene method. *Rylander-Rudqvist et al. Cancer Epidemiol Biomarkers Prev. : 15(9) (2006)*
(3) Nucleic Acid from Saliva and Salivary glands for Noninvasive Genotyping of Crohn's Disease patients *J.Hong et al. Genetic Testing : 12(4) (2008) : 587-589*
(4) Utilising the Oragene DNA self collection Kit for the isolation of DNA from saliva for HLA typing by SBT and Luminex beads. *D.Whittle et al. Hum. Imm. : 69 (Supp 1) (2008)*

MK-00087 Issue 1/2012-05

BE A MATCH, SAVE A LIFE