ORAcollect®•DNA

HLA typing using DNA from oral samples collected with ORAcollect®•DNA

Traditionally, marrow donor registries and transplant centers collect DNA samples from potential donors through either a blood sample or a buccal swab sample. Both of these sample types present some challenges as blood collections are invasive for the donor and swabs present time and sample quality issues. These challenges can be eliminated by using ORAcollect®•DNA to collect a DNA sample from saliva for HLA typing.

Unlike buccal swabs, samples collected with ORAcollect•DNA are liquid, bacteriostatic, yield larger amounts of DNA, and are easy to integrate into laboratory workflows. When collecting samples with buccal swabs, users are typically instructed to let the sample dry in the air prior to packaging it for transport in order to reduce bacterial growth. In addition, there is often a need to collect with multiple buccal swabs in order to compensate for the low quantity and quality of DNA obtained from a single buccal sample to meet the requirements of downstream testing applications. The process of applying barcodes for sample tracking to multiple swabs is burdensome, difficult to manipulate and prone to error.

ORAcollect•DNA is designed to maximize ease of use and reliability of oral sample collection for use in the clinic or in unsupervised settings. The collection protocol is quick and easy for the user to collect a sample without assistance, and no drying time is needed. The ergonomic design of an integrated tube and sponge allows for easy handling, and less likelihood of dropping the sample during the collection process. The elimination of a "drying time" plus collecting with a single sponge results in saving time when collecting the sample. In order to reduce potential errors, save time and simplify the sample management process, the ORAcollect•DNA OCR-100 collection kit includes barcoding on the tube. Another contributing factor to the high quality sample obtained from ORAcollect•DNA is the bacteriostatic reagent which inhibits growth of bacteria and stabilizes DNA from time of sample collection to processing.

ORAcollect•DNA addresses the labour intensive processing time, cost, and quality issues that laboratories experience when working with buccal swabs. The physical design and liquid sample inherent with ORAcollect•DNA enables a number of key advantages that streamline laboratory processing. Sample management and traceability is simplified with the ORAcollect•DNA OCR-100 since there is a single barcoded tube to trace within the laboratory. The handling of the sample is facilitated since there is no manual cutting of swab tips, no need to wash and spin down swabs, and the removal of the sponge occurs automatically when the lab personnel unscrews the tube cap due to the integrated sponge/cap design. The reduction of manual steps when processing the ORAcollect•DNA sample reduces the chance of errors and cross contamination. The ease of processing a liquid sample in a standardized tube format is appreciated not only in manual processing environments, but even more so in larger throughput environments where the OCR-100 is compatible for use with liquid handling robots. Room temperature (23°C) stability for up to 60 days maintains sample quality from collection time to processing, even in cases of elevated temperatures and extended post-collection storage time either due to transport conditions or processing backlog.

Oral samples collected using ORAcollect•DNA provide an easier, more reliable, and stable method for collection and laboratory processing of DNA for Sequence-Specific Oligonucleotide Probes (SSOP), and Sequence Based Typing (SBT) which are both commonly used for HLA typing of potential donors.

In the current study we examined the performance of ORAcollect•DNA samples using SSOP and SBT technologies. Samples from five different donors were collected in triplicate using the ORAcollect•DNA OCR-100 collection kit. DNA was extracted from two 500 µL aliquots of each sample using prepIT®•L2P as per the standard prepIT•L2P protocol[1].

Purified DNA was shipped to a large US-based CLIA lab[2] (hereafter referred to as Lab A) for HLA-typing on the two platforms. The Lab A report describes the successful HLA typing of ORAcollect•DNA samples using the two methods. In addition, the HLA calls in this study are concordant with results obtained previously from Oragene•DNA/saliva samples provided by the same donors[3].

For In Vitro Diagnostic Use

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ORAcollect•DNA OCR-100 sample study

Purpose

The purpose of this study was to determine the viability of performing HLA testing on samples purified from the ORAcollect•DNA OCR-100 kit using the established Lab A SSOP and SBT testing procedures. Lab A received five donor samples from DNA Genotek. These samples contained extracted DNA purified from the ORAcollect•DNA OCR-100 collection kit.

Procedure

Samples from five different donors were collected in triplicate. All samples were extracted by DNA Genotek who also provided DNA concentration data:

Sample	Tube	DNA concentration (ng/µL)	DNA yield (µg)
Sample HOC1	A	102.01	10.20
	B	132.75	13.28
	C	201.19	20.12
Sample HOC2	A	104.65	10.46
	B	92.28	9.23
	C	119.57	11.96
Sample HOC3	A	97.26	9.73
	B	150.94	15.09
	C	159.86	15.99
Sample HOC4	A	178.15	17.81
	B	166.59	16.66
	C	149.42	14.94
Sample HOC5	A	124.48	12.45
	B	125.15	15.52
	C	137.40	13.74

HLA testing was performed on all samples using two different methods. For all methods, the HLA-A, HLA-B, and HLA-DRB1 regions were typed. First, SSOP testing was performed using Lab A's in-house methodology and established procedures. Next, SBT was performed. Sequencing was done for all three loci using kits manufactured by Celera Diagnostics and distributed by Abbott Molecular. Electopherograms (EPG's) were generated using ABI genetic analyzers and read using Assign 3.5.1.45 software from Conexio Genomics. All results were based on the IMGT 3.3.0 database.

Raw data was gathered and checked for quality. Typings from both testing methods were compiled and checked for concordance then were combined to give a final HLA typing for each locus.

Results

After SSOP testing was completed, all typings were collected and coded. The SSOP typings are as follows:

SSOP results

Sample	HLA-A	HLA-A	HLA-B	HLA-B	HLA-DRB1	HLA-DRB1
HOC1	02:KPSV	23:KPST	39:NNUX	49:KFBJ	11:JNFZ	11:MTRY
HOC2	24:KVYG	68:KVZD	07:JYZA	27:JYKR	03:MJZK	15:JUFU
HOC3	01:MFFV	02:KRFH	08:KEZK	39:KFAX	01:01	03:MJZK
HOC4	02:KRFJ	-	15:KETM	44:KDKA	04:01	12:JUFV
HOC5	03:JZAV	33:JYFG	07:JVZB	14:JVRU	01:02	15:JUFU

Data quality for all samples was very good. The dot intensity from positive reactions was clear for all samples and matched that of the internal Lab A controls. Typings were obtained for all samples.

Once SBT testing was completed, all typings were collected and coded. The SBT typings are as follows:

SBT results

Sample	HLA-A	HLA-A	HLA-B	HLA-B	HLA-DRB1	HLA-DRB1
HOC1	02:CEZE	23:BRXU	39:BMFM	49:01	11:JVDN	11:04
HOC2	24:WYU	68:01	07:ANVB	27:EKN	03:CYSJ	15:01
HOC3	01:BMMP	02:ANGA	08:01	39:BMFM	01:JHMB	03:CYSJ
HOC4	02:ANGA	-	15:GYF	44:AMUT	04:01	12:DUKV
HOC5	03:XKS	33:01	07:ANVB	14:02	01:02	15:01

For the HLA-A and HLA-B loci, exons 2, 3, and 4 were sequenced in the forward and reverse direction. For the DRB1 region, exon 2 was sequenced in the forward and reverse direction. This constitutes the first layer of sequencing for each sample. For some donors, Heterozygous Ambiguity Resolution Primers (HARPs) were used to sequence a single allele in samples with

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cis/trans ambiguities. Similarly, one sample underwent a second PCR with group specific primers to eliminate ambiguous results. The data quality for all reactions could best be expressed through assigned base call scores (BCS) for each sample. The Assign software gives each sample a BCS based on the average signal intensity, signal to noise ratio, alignment, and peak characteristics. These scores are between 0 and 100 with 100 being the best quality. The BCS of each sample for the first layer of sequencing are as follows:

SBT BCS

Sample	HLA-A	HLA-B	HLA-DRB1
HOC1	86.81265207	78.57177616	79.78481013
HOC2	86.84428224	88.1459854	77.97046414
HOC3	85.61800487	86.21776156	81.10548523
HOC4	89.62530414	86.69221411	83.1814346
HOC5	86.00729927	79.86374696	79.07594937

BCS for all samples are well within the normal range.

Once all testing was completed, the results from both the SSOP and SBT methods were combined and a final HLA typing was produced for all samples. The combined typings are as follows:

Combined results

Sample	HLA-A	HLA-A	HLA-B	HLA-B	HLA-DRB1	HLA-DRB1
HOC1	02:ANGA	23:CJT	39:BMFM	49:01	11:04	11:CTPB
HOC2	24:WYU	68:01	07:ANVB	27:EKN	03:01	15:01
HOC3	01:BMMP	02:ANGA	08:01	39:BMFM	01:01	03:01
HOC4	02:ANGA	-	15:GYF	44:AMUT	04:01	12:DUKV
HOC5	03:XKS	33:01	07:ANVB	14:02	01:02	15:01

These final results meet the classification of high resolution typing set forth in the 2007 manuscript by Cano et al.

Conclusions

The five DNA samples purified with the ORAcollect•DNA OCR-100 collection kit were successfully typed using SSOP and SBT procedures. All SSOP data and results were of very good quality. Similarly, the sequencing data that was produced showed very good signal quality and strength. No reactions were repeated for SSOP testing, and only one out of eighty-eight total sequence reactions had to be repeated. This reaction involved a HARP that was selected to eliminate a cis/trans ambiguity but failed to produce adequate signal. The reaction was repeated successfully.

HLA-A, HLA-B, and HLA-DRB1 typings were reached for all samples. The results obtained through SSOP testing were concordant with results obtained from SBT. Data and results from both testing methods were successfully combined to yield a high resolution typing for each locus based on the 2007 publication by Cano et al[4].

Based on these results, the extracted DNA submitted by DNA Genotek that was purified from the ORAcollect•DNA OCR-100 collection kit is viable for HLA testing using the procedures established at Lab A.

References

1 Laboratory protocol for manual purification of DNA from 0.5 mL of sample. DNA Genotek. PD-PR-006.
2 In accordance to confidentiality agreements, reference to the third party lab responsible for the report has been removed.
3 HLA typing using saliva DNA collected with Oragene. DNA Genotek. PD-WP-020.
4 Cano P., et al. (2007). Common and Well-Documented HLA Alleles: Report of the Ad-Hoc Committee of the American Society for Histocompatibility and Immunogenetics. *Human Immunology*. 68, 392-417.

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