ORAgene®·DNA

HLA typing using saliva DNA collected with Oragene®

Traditionally, HLA registries and donor recruitment centers collect DNA samples from volunteers and potential donors through either a blood sample or a buccal swab sample. Both of these sample collection methods pose various challenges that can be eliminated using Oragene® to collect DNA samples from saliva for both low resolution and high resolution HLA typing.

Saliva samples collected using Oragene provide an easier, more reliable and stable method for collecting DNA for all HLA typing technologies.

Unlike blood samples, Oragene allows volunteers and potential donors to provide a saliva sample without assistance, anywhere and at anytime. For example, a volunteer can provide a reliable sample at home and mail the sample back through the regular postal system. By eliminating the need for a trained phlebotomist, the registry improves convenience for the volunteer and dramatically reduces the cost and complexity of the entire process. Saliva collection simplifies the recruitment process and increases donor participation, therefore maximizing the likelihood of finding life-saving donor/recipient matches.

Oragene provides a superior DNA sample compared to buccal swabs, with a collection protocol that is easy and painless. Oragene stabilizes DNA from saliva for at least 5 years at ambient temperatures allowing registries a cost-effective banking option that ensures integrity of the DNA sample without degradation. Saliva samples collected with Oragene also produce a high quantity and quality of DNA allowing the HLA typing center to use this sample type for high resolution confirmatory testing.

In the current study we examined the performance of Oragene samples using three technologies commonly used for HLA typing: Sequence-Specific Oligonucleotide Probes (SSOP), Sequence Based Typing (SBT), and Sequence Specific Primers (SSP). In addition we investigated the robustness of samples collected using the Oragene kits. Freshly collected Oragene/saliva samples were compared to Oragene/saliva samples collected from the same individuals under the conditions stated below:

- 3 freeze/thaw cycles (-20°/+50°C)

or

- Ambient temperature storage (~23°C) for 5 years.

The Oragene/saliva samples were purified using the provided reagents and manual purification protocol using prepIT®•L2P. The samples were collected, stored, purified and coded by DNA Genotek Inc. Purified DNA was shipped to a large US-based CLIA lab† (hereafter referred to as Lab A) for analysis on the three platforms. The Lab A report describes the successful HLA typing of Oragene/saliva samples using the three methods described and demonstrates that long-term storage or extreme temperature fluctuations produce successful typing results on both low and high resolution technologies as well as freshly collected Oragene/saliva samples.

† In accordance to confidentiality agreements reference to the third party lab responsible for the report has been removed.

For In Vitro Diagnostic Use

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DNA Genotek sample study Oragene HLA validation

With input from DNA Genotek where specified 07/19/10

Purpose

LAB A received DNA samples purified from Oragene collected saliva samples (DNA Genotek). Three different variations of this sample type from five different donors were sent. LAB A also included two in-house control donor samples in the form of buccal swabs. The purpose of this study was to determine the viability of performing HLA testing on Oragene collected saliva samples using the established testing procedures of LAB A's Testing Division.

Procedure

Samples from five different donors were sent. The five donors were labeled HOG1, HOG2, HOG3, HOG4, and HOG5. For each donor, three variations of the Oragene collected sample type was received. All sample type variations arrived as extracted DNA. To these samples, LAB A added two internal buccal swab controls; QC22 and QC13. Full sample listing is as follows:

Sample	Sample type
HOG'#'A	Oragene sample
HOG'#'B	Oragene sample stored at room temperature for over 5 years
HOG'#'C	Oragene sample subjected to 3 freeze (-20°C) / thaw (50°C) cycles
QC22	Buccal control
QC13	Buccal control

***Note**: Information in this table provided by DNA Genotek.*

First, the two buccal controls were extracted using Qiagen spin columns from Qiagen's blood mini kit. Once this extraction was complete, the DNA concentrations of all samples were measured using a NanoDrop spectrophotometer:

Initial concentrations

Sample	ng/µL	260/280
HOG1A	137.30	1.73
HOG1B	171.14	1.85
HOG1C	209.11	1.93
HOG2A	143.80	1.67
HOG2B	52.41	1.81
HOG2C	134.89	1.71
HOG3A	180.38	1.89
HOG3B	110.85	1.85
HOG3C	226.34	1.88
HOG4A	143.66	1.76
HOG4B	106.90	1.85
HOG4C	207.06	1.83
HOG5A	219.69	1.82
HOG5B	185.04	1.92
HOG5C	349.53	1.89
QC22	14.36	1.69
QC13	13.03	1.81

HLA testing was performed on all samples using three 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. EPG's were generated using ABI genetic analyzers and read using Assign 3.5+ software from Conexio Genomics. Finally, SSP testing was performed on all samples for a single HLA-B allele using Invitrogen SSP kits. All results were based on the IMGT 2.24 database.

Results from all testing methods were compiled and checked for concordance. Finally, raw data was analyzed for quality differences between the three variations of the Oragene collected saliva samples.

Results

After SSOP testing was completed, all typings were recorded and checked for concordance between the sample types of the different donors. The SSOP typings are as follows:

SSOP results

Sample	HLA-A	HLA-A	HLA-B	HLA-B	HLA-DRB1	HLA-DRB1
HOG1A	02:XX	23:XX	39:XX	49:0101	11:01	11:04
HOG1B	02:XX	23:XX	39:XX	49:0101	11:01	11:04
HOG1C	02:XX	23:XX	39:XX	49:0101	11:01	11:04
HOG2A	24:XX	68:XX	07:XX	27:XX	03:01	15:01
HOG2B	24:XX	68:XX	07:XX	27:XX	03:01	15:01
HOG2C	24:XX	68:XX	07:XX	27:XX	03:01	15:01
HOG3A	01:XX	02:XX	08:XX	39:XX	01:01	03:01
HOG3B	01:XX	02:XX	08:XX	39:XX	01:01	03:01
HOG3C	01:XX	02:XX	08:XX	39:XX	01:01	03:01
HOG4A	02:XX	–	15:XX	44:XX	04:01	12:DUKV
HOG4B	02:XX	–	15:XX	44:XX	04:01	12:DUKV
HOG4C	02:XX	–	15:XX	44:XX	04:01	12:DUKV
HOG5A	03:XX	33:XX	07:XX	14:XX	01:02	15:01
HOG5B	03:XX	33:XX	07:XX	14:XX	01:02	15:01
HOG5C	03:XX	33:XX	07:XX	14:XX	01:02	15:01
QC22	02:XX	–	44:XX	57:XX	07:01	11:01
QC13	02:XX	03:XX	07:XX	27:XX	08:01	15:01

Data quality for all samples was very good. The dot intensity from positive reactions was clear for all Oragene collected saliva samples. Typings were obtained for all samples.

Once SBT testing was completed, all typings were recorded and checked for concordance between the sample types of the different donors. The SBT typings are as follows:

SBT results

Sample	HLA-A	HLA-A	HLA-B	HLA-B	HLA-DRB1	HLA-DRB1
HOG1A	02:CEZE	23:BRXU	39:DAUV	49:BAXH	11:BCP	11:04
HOG1B	02:CEZE	23:BRXU	39:DAUV	49:BAXH	11:BCP	11:04
HOG1C	02:CEZE	23:BRXU	39:DAUV	49:BAXH	11:BCP	11:04
HOG2A	24:BSMU	68:BDDJ	07:ANVB	27:EKN	03:01	15:01
HOG2B	24:BSMU	68:BDDJ	07:ANVB	27:EKN	03:01	15:01
HOG2C	24:BSMU	68:BDDJ	07:ANVB	27:EKN	03:01	15:01
HOG3A	01:CENZ	02:CCCZ	08:TM†	39:BZDK†	01:AH	03:01
HOG3B	01:CENZ	02:CCCZ	08:01	39:BMFM	01:AH	03:01
HOG3C	01:CENZ	02:CCCZ	No type	No type	01:AH	03:01
HOG4A	02:ANGA	–	15:CTFG	44:CTFF	04:01	12:DUKV
HOG4B	02:ANGA	–	15:CTFG	44:CTFF	04:01	12:DUKV
HOG4C	02:ANGA	–	15:CTFG	44:CTFF	04:01	12:DUKV
HOG5A	03:XKS	33:01	07:ANVB	14:02	01:02	15:01
HOG5B	03:XKS	33:01	07:ANVB	14:02	01:02	15:01
HOG5C	03:XKS	33:01	07:ANVB	14:02	01:02	15:01
QC22	02:ANGA	–	44:AMUT	57:01	07:01	11:BCP
QC13	02:ANGA	03:XKS	07:DEZW	27:EHNR	08:01	15:01

† *HARP failed.*

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. For some donors, Heterozygous Ambiguity Resolution Primers (HARPs) were used to sequence a single allele in samples with cis/trans ambiguities. 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 and HARP 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 and corresponding HARPs are as follows:

SBT BCS

Sample	HLA-A	HARP	HLA-B	HARP	HLA-DRB1	Group 2	HARP
HOG1A	87.413625	43.176211	85.261557		85.590717		42.53158
HOG1B	86.540146	42.709251	80.976886		81.223629		42.52105
HOG1C	86.951338	43.797357	84.380779		85.945148		42.04211
HOG2A	87.420925		85.972019	43.165217	79.662447	84.763713	
HOG2B	87.087591		86.006083	43.130435	79.126582	80.64557	
HOG2C	87.519465		85.07056	42.843478	86.059072	86.092827	
HOG3A	86.051095		83.690998	0	81.485232	85.227848	
HOG3B	86.332117		84.913625	29.725664	82.160338	85.118143	
HOG3C	86.53163		16.305147	0	84.616034	87.172996	
HOG4A	89.190998		68.391727	30.360947	87.56962	81.860759	
HOG4B	89.473236		86.104623	42.805882	81.320675	86.008439	
HOG4C	89.20073		86.187348	42.882353	80.400844	85.303797	
HOG5A	86.541363		84.585158	42.327511	86.151899	82.835443	
HOG5B	87.153285		83.718978	41.148472	79.64135	84.493671	
HOG5C	86.80292		81.363747	37.495652	85.021097	85.755274	
QC22	88.875912		80.998783		85.409283	83.417722	
QC13	85.675182	43.806167	85.762774		71.85654	85.561181	

BCS for most samples are well within the normal range. Please note however, that HARP scores were always lower due to being sequenced in only a single direction. The HARP for HOG3A and HOG3C failed. Also, the general sequence for HOG3C failed.

A single HLA-B SSP kit was run to type a single HLA-B allele for each sample. Once testing was completed, all typings were recorded and checked for concordance between the sample types of the different donors and controls. The SSP typings are as follows:

SSP results

Sample	HLA-B
HOG1A	39:01
HOG1B	39:01
HOG1C	39:01
HOG2A	07:02
HOG2B	07:02/07:44†
HOG2C	07:02
HOG3A	39:01
HOG3B	39:01
HOG3C	39:01
HOG4A	44:02
HOG4B	44:02
HOG4C	44:02
HOG5A	07:02
HOG5B	07:02
HOG5C	07:02
QC22	No type
QC13	07:02

† Single lane failure, unrelated to sample quality.

Data quality was very good for nearly all samples. This assessment was based on the presence and intensity of all positives and all positive control bands. Also taken into account was the absence of bands from any non-specific priming. Eight lanes or positive control bands failed for QC22 and 1 failed for HOG2B. Aside from these failures, band intensity and presence for all positives and positive control bands was excellent for all samples. For every sample, there was little or no evidence of bands showing non-specific priming.

Conclusions

After compiling all data, result concordance was checked. Results for each donor with all sample type variations were concordant across all three tests. Both LAB A internal QC samples matched expected typings for all tests.

The SSOP data quality was very good for all variations of the Oragene collected saliva samples.

The SBT data quality was very good for all sample variations of HOG1, 2, 4, and 5. For HOG3A and HOG3C, the HARP reaction failed. Furthermore, HOG3C failed completely while HOG3B worked well for the general sequence as well as the HARP. The reason for these failures could not be easily determined. More testing would be needed to determine the root cause. For the purpose of this study, it is important to note that the data quality matched between all variations of donors HOG1, 2, 4, and 5.

The SSP data quality was excellent for all sample variations of the Oragene collected saliva samples. One lane failed for variation B for donor HOG2 causing the inclusion of one additional allele possibility, however, this is a very common occurrence with SSP testing. Data quality between variations matched one another, and was equal to or better than that of the LAB A controls. This is best exhibited by the fact that LAB A QC22 failed for this test.

Based on these results, the extracted DNA of the three tested variations of Oragene collected saliva samples, Oragene/saliva is viable for HLA testing using the procedures established at LAB A's Testing Division.

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