HLA typing with QTYPE® 11 using DNA extracted from saliva

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INTRODUCTION

Human leukocyte antigen (HLA) matching is of vital importance to the success of solid organ and hematopoietic stem cell transplantation. QTYPE® 11 is currently the fastest commercial real-time PCR assay for HLA typing, allowing for the identification of alleles from HLA-A, -B, -C, -DRB1, -DRB3, -DRB4, -DRB5, -DQA1, -DQB1, -DPA1 and -DPB1 loci, with low-to-intermediate resolution in less than one hour. HLA typing with QTYPE 11 is validated for use with DNA extracted from whole blood, but until now has not been performed with DNA extracted from saliva. Collection of saliva is an easy, harmless and non-invasive procedure, without the need for a trained phlebotomist or hospital staff. This makes saliva an easily accessible and less hazardous biological material for DNA extraction and HLA typing.

MATERIAL & METHODS

24 saliva samples (de-identified) were collected, using 2 ml self-collection tubes (Oragene•DNA|OG-600 (DNA Genotek). The donors did not eat, drink, chew gum or smoke 30 minutes prior to the saliva collection. DNA from the 24 saliva samples were extracted using prepIT•L2P DNA extraction reagent (DNA Genotek) according to manufacturer instructions. The isolated DNA samples were quantified before diluted into a working concentration of 10 ng/µl. The saliva DNA was subsequently HLA typed using QTYPE 11 (LOT no. E042, CareDx AB, Stockholm, Sweden). 4.3 µg of DNA was used per QTYPE 11 assay, with 10 ng of DNA as input material per reaction. The QTYPE 11 assays were run on either a LightCycler® 480 II (Roche) or ViiA 7 (Thermo Scientific) real-time PCR instrument. The exported data were analyzed by the interpretation software SCORE™ 6 (v6.1.2, CareDx AB). In order to confirm that correct HLA typing of the saliva DNA samples was achieved, next-generation sequencing by AlloSeq® Tx (CareDx Pty Ltd, Fremantle, Australia) was used, see workflow in Figure 1.

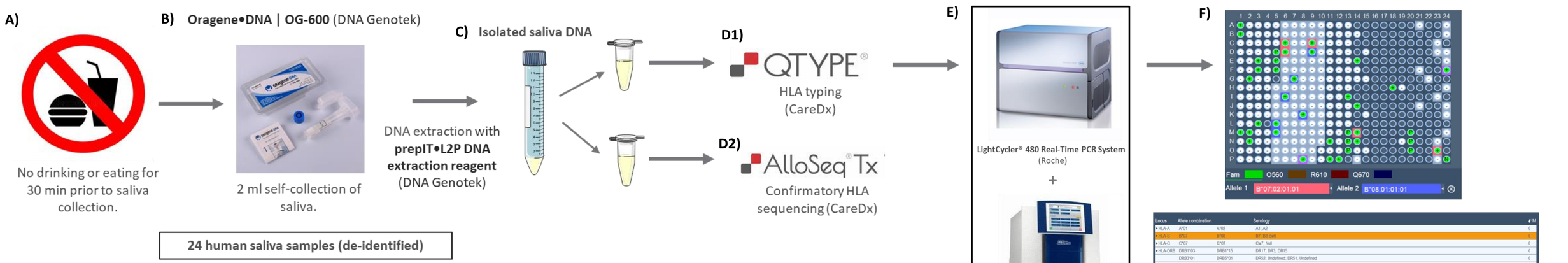

Figure 1. Experimental workflow - From sample collection to HLA typing results with QTYPE 11. A) The saliva donors did not eat, drink, chew gum or smoke for 30 min before sample collection. B) The donors collected the saliva samples themselves into 2 ml self-collection tubes. C) DNA was extracted from all 24 saliva samples. The isolated DNA was used for two purposes: D1) HLA typing with QTYPE 11, D2) Confirmatory HLA sequencing with AlloSeq Tx. E) 24 × QTYPE 11 assays (one per sample) were performed on real-time PCR instruments. F) To obtain HLA typing of each sample, raw data was exported and analyzed with the interpretation software SCORE 6.

METHOD – QTYPE 11

The 24 DNA samples extracted from saliva were typed using the QTYPE 11 assay. QTYPE 11 identifies 11 loci of the HLA gene complex located in chromosome 6, namely HLA-A, -B, -C, -DRB1/3/4/5, -DQA1, -DQB1, -DPA1 and -DPB1 (as highlighted with green in Figure 2A) with low-to-intermediate resolution, in less than one hour. QTYPE 11 is a TaqMan® probe-based quantitative polymerase chain reaction (qPCR) assay (Figure 2B) with pre-aliquoted reaction mixes in a 384-well plate layout. Each well contains of reaction mixes consisting of dried sets of target-specific primer pairs and fluorescent reporter probes that recognizes and binds a subset of HLA alleles (Figure 2C).

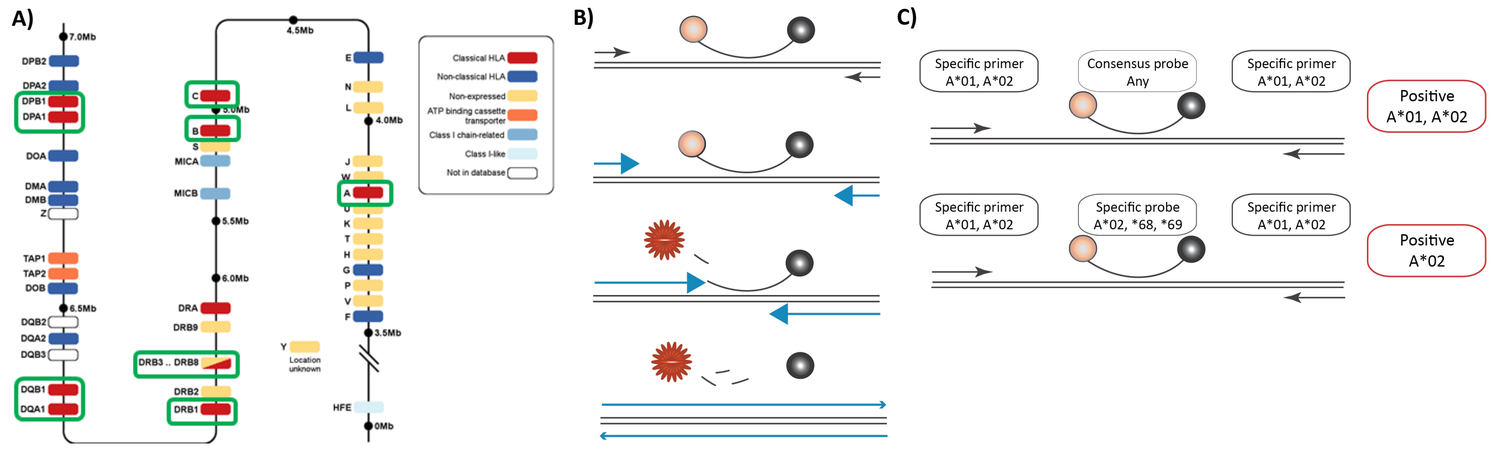

Figure 2. The HLA loci targeted by QTYPE 11 and the principle of the QTYPE 11 assay. A) QTYPE 11 identifies 11 HLA loci (marked in green) with low-to-intermediate resolution. B) QTYPE 11 is a TaqMan probe-based qPCR assay. C) The QTYPE 11 assay is a 384-well plate containing dried multiplexed reaction mixes that consists of target-specific primers and reporter probes.

Sample name	DNA concentration (ng/µl)	$OD_{260/280}$ ratio
SAL02	298,8	1,89
SAL03	389,0	1,70
SAL05	405,4	1,75
SAL06	581,6	1,86
SAL07	208,3	1,75
SAL09	422,4	1,87
SAL10	1148,0	1,78
SAL11	844,2	1,86
SAL12	527,0	1,85
SAL13	434,3	1,83
SAL14	443,4	1,80
SAL15	918,5	1,79
SAL16	537,0	1,82
SAL17	464,1	1,66
SAL18	638,1	1,86
SAL19	877,6	1,81
SAL20	274,1	1,82
SAL21	350,8	1,86
SAL22	872,1	1,88
SAL23	181,7	1,84
SAL24	798,2	1,71
SAL25	235,5	1,70
SAL27	391,2	1,77
SAL28	107,0	1,85

Table 1. DNA concentration and $OD_{260/280}$ ratio from saliva samples.

A1)

Locus	*Genotype*		*Serological eq.*
HLA-A	A*11	A*26	A11; A26, A10
HLA-B	B*38	B*40	B38; B60 Bw4/Bw6
HLA-C	C*03	C*12	Cw10; Undefined
HLA-DRB	DRB1*11	DRB1*13	DR11; DR13
	DRB3*01	DRB3*02	DR52, Undefined
HLA-DQA1	DQA1*01:03	DQA1*05	
HLA-DQB1	DQB1*03	DQB1*06:03	DQ7; DQ6
HLA-DPA1	DPA1*01:03		
HLA-DPB1	DPB1*02:01	DPB1*04:01	

A2)

HLA-A

Allele results

Group	Allele	tolerance range: (0)	Serological eq.
A*11	**A*11:01:01:01**	11:01:01:02-11:01:10, 11:01:12, 11:01:13, 11:01:15, 11:01:18-11:01:20, 11:01:22-11:01:27, 11:01:29-11:01:43, 11:01:45-11:01:60, 11:01:63, 11:01:64, 11:01:66-11:01:76, 11:01:79-11:01:93, 11:01:95-11:01:99, 11:06, 11:07, **11:09**, **11:12**, 11:23, 11:24:01, 11:26, **11:29**, **11:30**, 11:32:01-11:33:02, 11:36, 11:37, 11:41-11:49, 11:51, 11:52Q, 11:54:01-11:56, 11:58, 11:62-11:68, 11:71-11:76, 11:79, 11:81-11:83, 11:85-11:89, 11:91:01-11:93, 11:95-11:97, 11:100, 11:102, 11:104, 11:105, 11:107, 11:108, 11:111, 11:114, 11:117, 11:118, 11:120, 11:122-11:126, 11:128, 11:129, 11:131-11:136, 11:138, 11:139, 11:142, 11:143, 11:145, 11:146, 11:148-11:151, 11:153:01-11:154, 11:159-11:161, 11:163, 11:166-11:169, 11:171-11:174, 11:177, 11:179, 11:184, 11:185, 11:187-11:189, 11:192-11:200, 11:202-11:207, 11:212-11:214, 11:217-11:223, 11:225, 11:227-11:232, 11:234, 11:235Q, 11:239-11:247, 11:249, 11:252-11:254, 11:258-11:261, 11:263, 11:265-11:268, 11:270, 11:272Q-11:280, 11:283-11:286, 11:289, 11:291-11:296, 11:298-11:307, 11:309, 11:311, 11:313Q-11:324, 11:327-11:333, 11:337-11:339, 11:341-11:344, 11:349, 11:350, 11:351Q-11:354	Null
	A*11:69N, 11:99N, 11:109N, 11:115N, 11:127N, 11:137:01N, 11:137:02N, 11:180N, 11:208N, 11:215N, 11:238N, 11:251N, 11:287N, 11:340N, 11:347N		
A*26	**A*26:01:01:01**	26:01:01:02, 26:01:01:04-26:01:02, 26:01:04-26:01:13, 26:01:15-26:01:20, 26:01:22-26:01:61, 26:01:63, **26:02:01**, 26:02:02, **26:08:01:01**, 26:08:01:02, 26:08:03, **26:12**, **26:15-26:17**, **26:20**, 26:22-26:24, 26:26, 26:27, **26:31**, 26:32, 26:35-26:42, 26:43:02-26:48, 26:50-26:59, 26:61-26:70, 26:74-26:77, 26:79-26:90, 26:94-26:102, 26:104-26:106, 26:108-26:110, 26:113-26:126, 26:128, 26:129, 26:131-26:137, 26:139-26:144, 26:147-26:160, 26:162-26:165, 26:166Q-26:171, 26:173-26:175, 26:178, 26:179, 26:181-26:183, 26:185-26:190, 26:192, 26:194	A26, -, A10
	A*26:01:01:03N, 26:25N, 26:60N, 26:71N, 26:107N, 26:127N, 26:145N, 26:161N, 26:180N, 26:191N	Null	

HLA-A

B)

HLA-A	HLA-A	HLA-B	HLA-B	HLA-C	HLA-C
11:01:01:01	26:01:01:01	38:01:01:01	40:01:02:04	03:04:01:01	12:03:01:01

HLA-DRB1	HLA-DRB1	HLA-DRB3	HLA-DRB3	HLA-DRB4	HLA-DRB4
11:01:01	13:01:01	01:01:02:02	02:02:01:02		

HLA-DRB5	HLA-DRB5	HLA-DQA1	HLA-DQA1	HLA-DQB1	HLA-DQB1
		01:03:01	05:05:01	03:01:01:03	06:03:01:01

HLA-DPA1	HLA-DPA1	HLA-DPB1	HLA-DPB1
01:03:01:01	01:03:01:04	02:01:02	04:01:01

C)

HLA locus	Number of samples	Concordant	Discordant	Overall agreement
A	24	24	0	100%
B	24	24	0	100%
C	24	24	0	100%
DRB1	24	24	0	100%
DRB3	24	24	0	100%
DRB4	24	24	0	100%
DRB5	24	24	0	100%
DQA1	24	24	0	100%
DQB1	24	24	0	100%
DPA1	24	24	0	100%
DPB1	24	24	0	100%

Table 2. Concordance between QTYPE 11 and AlloSeq Tx typing results. A1) Summarized SCORE 6 report for sample SAL20. A2) List of possible HLA-A allele results for SAL20 suggested by SCORE 6. B) Summarized HLA typing results by AlloSeq Tx for sample SAL20. C) Concordance between HLA typing by QTYPE 11 and AlloSeq Tx.

RESULTS

Good DNA yield (21.4-287 µg) was obtained from the saliva samples with sufficient DNA to run a minimum of 4 × QTYPE 11 assays and on average 29 × QTYPE 11 assays. Purity ($OD_{260/280}$ ratio between 1.66-1.89) of the saliva DNA samples were comparable to DNA extracted from blood samples (average $OD_{260/280}$ ratio of 1.67-2.0) in a previous clinical performance evaluation study for QTYPE 11. All 24 saliva DNA samples were successfully HLA typed with QTYPE 11 and typing results were fully concordant with HLA sequencing by AlloSeq Tx. As QTYPE 11 have a low-to-intermediate (1st to 2nd field) typing resolution, results were considered concordant if any of the allele results suggested by SCORE 6 were the same as reported by AlloSeq Tx in higher resolution (see example for sample SAL20 in Table 2), for all 11 HLA loci tested.

CONCLUSIONS

This is the first time that HLA typing with DNA extracted from saliva has been performed with QTYPE 11. With these promising preliminary results, we aim to validate QTYPE 11 as an HLA typing assay that can be used with saliva, as a non-invasively collected sample type. Saliva samples can be collected from the comfort of an individual's home, without the need for hospital staff. This increases the convenience of transplant matching for the donor and recipient. Here in this pilot study, we show the following:

- Fast, accurate HLA typing with saliva DNA for the first time using QTYPE 11
- Increased accessibility and ease of HLA typing with QTYPE 11 – saliva samples are a good alternative to blood samples
- DNA extracted from saliva makes HLA typing with QTYPE 11 a non-invasive procedure
- Saliva is a viable source of quality DNA for use with QTYPE 11, but further validation is required before the use of saliva can be approved for routine in vitro diagnostic use

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