HLA typing with QTYPE® 11 using DNA extracted from saliva

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 min prior to saliva collection. DNA from the 24 saliva samples were extracted using prepTi®2DP 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 VIA 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 with AlloSeq® Tx (CareDx Pty Ltd, Fremantle, Australia) was used, see workflow in Figure 1.

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).

RESULTS

Good DNA yield (21.4-287 μg) was obtained from the saliva samples with sufficient DNA to run a minimum of 4 x QTYPE 11 assays and on average 29 x QTYPE 11 assays. Purity (OD260/280 ratio between 1.66-1.89) of the saliva DNA samples were comparable to DNA extracted from blood samples (average OD260/280 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 discordant 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.