New Data Analysis Method to Detect Loss of HLA by One Lambda LABType Products

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Abstract

One Lambda LABType products combining the robustness of reverse-sequence-specific oligonucleotide (rSSO) typing method with the Luminex platform are commonly used to obtain HLA typing in preparation for transplantation. Now we demonstrated feasibility to use LABType products to detect loss of HLA alleles by novel data analysis method. To evaluate our new algorithm, we used mixtures of DNA imitating different levels of chimerism in haplo-matched or mismatched transplants, and different levels of HLA loss for “patient” haplotype. Our tests demonstrated feasibility to detect complete or partial loss of HLA in samples with “chimerism” level of at least 10% and total DNA input at least 5 ng per reaction.

Introduction

One therapeutic option for patients suffering from high-risk acute myeloid leukemia is allogeneic hematopoietic stem cell transplantation. Relapses after transplantations are still frequent and can cause mortality in transplant patients.

There are different genomic mechanisms that allow leukemia cells to evade the donor immune system causing a relapse of the disease. This includes genomic loss of HLA alleles (~30% in haploidentical transplants and 5-15% in unrelated transplants). Donor lymphocyte infusion (DLI) commonly used post transplant will not provide expected benefits for patients suffering from relapse affected by loss of HLA. Depending on the mechanisms of relapse and immune evasion, a clinical decision must be made to change treatment or select an alternative donor. Identifying loss of heterozygosity (LOH) during relapses remains critical for patient care.

Monitoring levels of chimerism and minimal residual disease (MRD) are standard of care in HSCT patients. Changes in chimerism levels or increased MRD indicate a possible relapse, which may progress through one of the immune evasion pathways. Ability to detect loss of HLA at earlier stages of relapse may help to select an appropriate treatment, in particular, when new transplant is required.

Our team investigated feasibility to develop new analysis method detecting loss of HLA utilizing data generated by One Lambda LABType products with Labscan 100 and FM3D instruments.

Methods and materials

We tested two homozygous DNA samples 9040 and 9058 and their mixes in several different ratios imitating possible loss of one or another allele (Table 1) with One Lambda LABType products for several loci – A, B, C, and DRB1.

Table1. Homozygous DNA ratios mimicking LOH

Sample Mixture	Ratio						
9040:9058	1:1	1:2	1:5	1:10	2:1	5:1	10:1

We also tested different ratios of homozygous and heterozygous DNA mixtures to mimic both chimerism and LOH (Table 2).

Table 2. Homozygous and heterozygous DNA mixtures mimicking chimerism with LOH (represented by minor allele - allele unique for patient)

Sample	Typing	Allele (Unique or Common for Patient and Donor)	Represent	Minor Allele	Chimerism (Patient vs Donor)		
9040	B*49:01:01	Common	Patient	100%	10%	20%	50%
9058	B*45:01:01	Unique for patient					
E20113	B*49:01:01 B*15:01:01	Common Unique for donor	Donor				
9040	B*49:01:01	Common	Patient	10%			
9058	B*45:01:01	Unique for patient					
E22237	B*49:01:01 B*15:01:01	Common Unique for donor	Donor				
9040	B*49:01:01	Common	Patient	20%			
9058	B*45:01:01	Unique for patient					
E22237	B*49:01:01 B*15:01:01	Common Unique for donor	Donor				

We also used individual DNA samples to monitor background signals for each allele. Total DNA input per reaction is reflecting amount of DNA mix used for amplification with primers for each LABType product.

Results

We tested the RSSOA1 classic kit using different DNA inputs ranging from 1 ng to 20 ng. The 1 ng input showed significantly lower TMFI (trimmed mean fluorescence intensity) positive signals for unique markers (microspheres), although the normalized values did not differ significantly. Although the 1 ng input resulted in increased 95% confidential intervals, the kit was still able to detect allelic loss at this input level. Difference in allelic ratio is already noticeable with 1:2 ratio and is clearly significant when one of the alleles is reduced to 20% in the mix. Our new analysis method relies on data generated by beads unique for each allele. In total, 9 unique markers for 9040 and 11 unique markers for 9058 were identified by RSSOA1 classic kit in our mixes. Data from beads unique for each allele were analyzed to generate allele specific scores demonstrated on Figure 1.

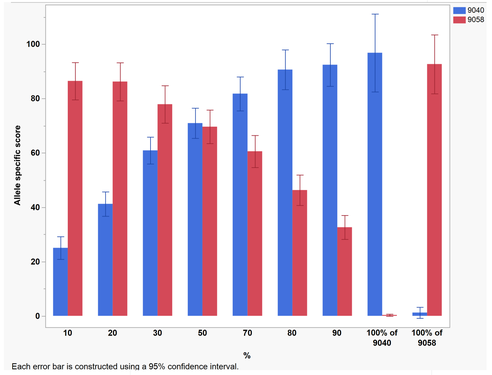
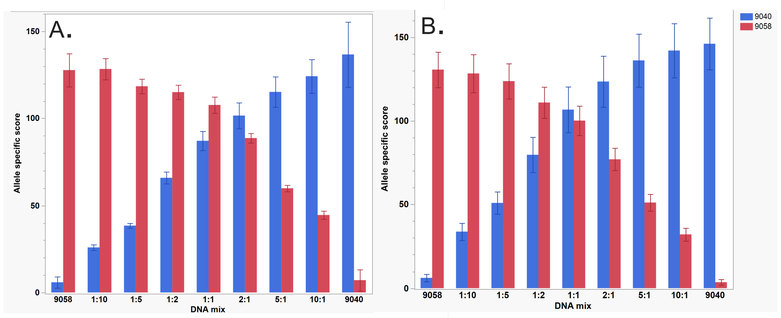


Figure 1. Data analysis for two homozygous DNA mix test

DNA mixes were analyzed by RSSOA1 product. The A locus classic kit contains 9 unique markers for sample 9040 and 11 unique markers for sample 9058. Total DNA input was 20 ng per reaction. X axis displays DNA ratios expressed in present values.

RSSO CWD kits provide higher level resolution and include more beads and thus can provide more unique markers per allele than RSSO classic kits. As expected, we demonstrated that an increase in the number of unique markers allows to generate more accurate data and quantify fraction of each allele with higher reliability, which may be particularly impactful for certain allelic combinations. For example, C locus allele combination of 9040 and 9058 provide 4 unique markers for sample 9040 and 7 unique markers for sample 9058 when RSSO classic kit is used. As result, 1:1 DNA mix demonstrates a small shift in allelic ratio and higher background (Fig. 2A). Nevertheless, the C locus CWD kit provides 12 unique markers for 9040 and 18 unique markers for 9058 demonstrating significant improvement in allele specific scores generated for the same mixes (Fig. 2B). Thus, increased number of beads provided in latest lots of CWD products may allow to estimate allelic ratios more accurately due to increased availability of unique markers per allele.

Figure 2. Comparing results for C locus classic kit (A) and C locus CWD kit (B)



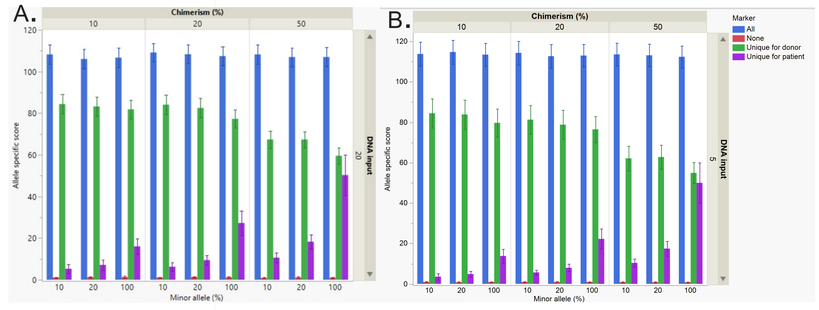
Since certain level of chimerism is expected for real life samples, we evaluated our analysis with DNA mixes imitating different level of chimerism. For this purpose, we mixed two homozygous DNA samples representing patient alleles and added another heterozygous DNA sharing alleles with one of them, thus imitating a haplo-matching transplant scenario. Combining these DNA samples in different ratios, we mimicked different levels of chimerism and different level of allelic loss. Example of experiment design with such DNA mixes is shown in Table 2.

The RSSOW1B CWD kit was tested using DNA inputs ranging from 1 to 20 ng, with various DNA mixtures mimicking LOH (from normal heterozygous containing 100% of unique patient allele to 10% of it remaining) and chimerism (10–50% of the patients’ alleles – both unique one and common with donor allele). Surprisingly, we did not see significant difference in quantification accuracy between total DNA inputs of 5 ng and 20 ng per reaction (Fig.3). Even with 1 ng of total DNA input, loss of HLA was detectable due to sufficient number of informative markers provided by B locus CWD kit. In addition to unique allele specific markers, we included into analysis beads specific for all alleles in the mix and beads representing background as an average negative for all tested DNAs.

Figure 3. Detecting loss of HLA in DNA mixes representing different levels of chimerism*

B CWD Lot 6 test was used. A. 20 ng total DNA input and B. 5 ng total DNA input per reaction

*Chimerism values in the graph show the fractions of the “patient’s” DNA



Increased number of alleles in chimeric mixture expected for haplo-transplants reduces number of allele specific markers available even with latest lots of CWD kits. To evaluate feasibility to increase number of useable markers in haplo-transplant samples, we explored combined analysis of multiple LABType products tested with single DNA mix. Such approach can be useable to enhance data analysis for limiting allelic combination in chimeric samples or when RSSO classic products are used.

Figure 4 shows combined analysis of B CWD and DRB1 CWD products tested with DNA mixes imitating various level of chimerism and HLA loss. Combined analysis of two loci data allows to improve HLA loss measurement even with 1 ng of DNA per reaction (Fig. 4A). No significant difference was observed between results obtained from 5 and 20 ng per reaction indicating that higher DNA input may only be needed for samples containing higher donor’s fractions.

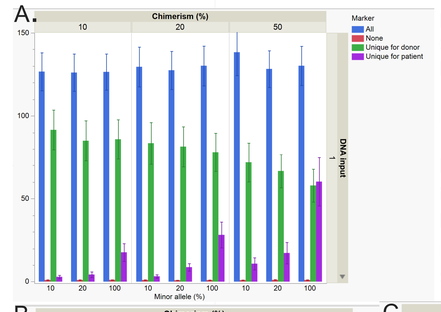
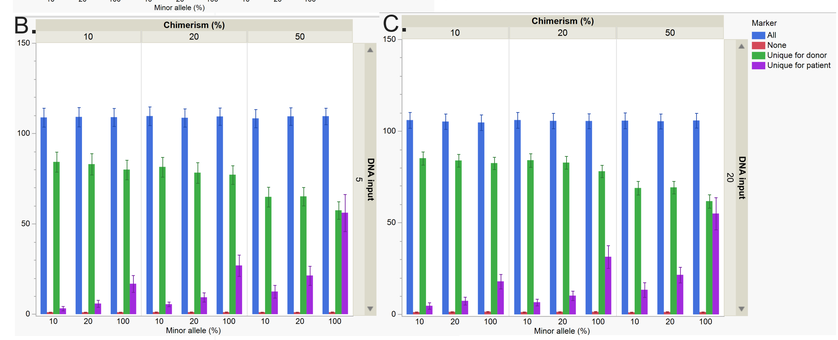


Figure 4. Combined analysis of B CWD and DRB1 CWD kits data

DNA mixes tested with (**A.**) 1 ng total input, (**B.**) 5 ng and (**C.**)20 ng per reaction.

Mixes were representing range from 10 to 50% of patient DNA per sample. Presence of unique patient allele was ranging from normal homozygous (100%) to 10% from original.



Conclusions

Preliminary data suggest feasibility of developing new method detecting complete or partial loss of HLA alleles using well established LABType technology. We performed multiple experiments using mixes of well-characterized DNA samples with known HLA typing. Feasibility data suggested that our new analysis algorithm may detect not only complete but also partial loss of HLA with DNA input starting from 1 ng per reaction. Our data showed that chimeric samples affected by complete or partial allelic loss can be analyzed by single LABType kit or data from multiple locus-specific kits can be analyzed in combination. Highest sensitivity to minor HLA allele fraction for low DNA input was achieved when multiple CWD kits were utilized in combined analysis. Using single CWD product may also be sufficient to accurately assess complete or partial loss of HLA, and we also demonstrated that useable data can be generated with SSO classic products providing an analysis options for up to 11 HLA loci.

Our data show a possibility for potential future application of LABType products in post-transplant monitoring of HSCT transplant patients.

This analysis method is experimental and has not been reviewed or approved by the FDA or other regulatory agencies. The new algorithm is still in feasibility stage and not commercially available.

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