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Title: Resolving incomplete single nucleotide polymorphism tagging of HLA-DQ2.2 for coeliac disease genotyping using digital droplet PCR

Short title: HLA-DQ2.2 genotyping with digital droplet PCR

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Abstract:

A hallmark of coeliac disease (CD) is the exceptionally strong genetic association with HLA-DQ2.5, DQ8, and DQ2.2. HLA typing provides information on CD risk important to both clinicians and researchers. A method that enables simple and fast detection of all CD risk genotypes is particularly desirable for the study of large populations. SNP-based HLA typing can detect the CD risk genotypes by detecting a combination of six SNPs but this approach can struggle to resolve HLA-DQ2.2, seen in 4% of European CD patients, due to the low resolution of one negatively predicting SNP. We sought to optimise SNP-based HLA typing by harnessing the additional resolution of digital droplet PCR to resolve HLA-DQ2.2. Here we test this two-step approach in an unselected sample of Mexican DNA and compare its accuracy to DNA typed using traditional exon detection. The addition of digital droplet PCR for samples requiring negative prediction of HLA-DQ2.2 enabled HLA-DQ2.2 to be accurately typed. This technique is a simple addition to a SNP-based typing strategy and enables comprehensive definition of all at-risk HLA genotypes in CD in a timely and cost-effective manner.

Keywords: Coeliac disease, digital droplet PCR, HLA, Mexican

Abbreviations: CD (coeliac disease), SNP (single nucleotide polymorphism), HLA (human leukocyte antigen).

1. Introduction

Coeliac disease (CD) is a highly prevalent and increasingly diagnosed autoimmune-like illness resulting from an aberrant immune response to dietary gluten (1). A hallmark of CD is the exceptionally strong genetic association with specific human leukocyte antigen (HLA)-DQ genotypes. These genotypes play a key role in pathogenesis by encoding major histocompatibility complex (MHC) class II heterodimers involved in gluten peptide presentation to pathogenic $CD4^+$ T cells (1). The strong HLA association in CD, and the clinical information that comes from identifying a specific HLA genotype, means that HLA typing for CD is an important and increasingly employed tool for researchers and clinicians.

In a large European cohort most CD patients (90%) expressed HLA-DQ2.5 (encoded by the DQA1*05 and DQB1*02 alleles) with the remainder expressing HLA-DQ8 (encoded by DQA1*03 and DQB1*03:02) or HLA-DQ2.2 (encoded by DQA1*02:01 and DQB1*02:02) (2). The presence of DQA1*05 without DQB1*02 (HLA-DQ7) is infrequently associated with CD and the CD risk conferred is likely to be very low (3, 4). HLA-DQ2.5 may be encoded in *cis* (e.g. HLA-DRB1*03-DQA1*05-DQB1*02) or in *trans* through the combination of HLA-DQ2.2 and HLA-DQ7 (e.g. DRB1*11-

DQA1*05-DQB1*03:01, DRB1*07-DQA1*02-**DQB1*02**). As the difference does not impact gluten peptide recognition, both confer a similar risk for CD. Notably, relative risk for CD development and also the clinical phenotype are affected by the HLA-DQ2.5 "gene-dose", a phenomenon that relates to the number of ± and ² heterodimers that can form and present gluten to T cells. The higher gene-dose conferred by HLA-DQ2.5 homozygosity compared to the heterozygous form is reflected by stronger $CD4^+$ T cell responses to gluten (5). For this reason, methods that detect HLA homozygosity are more informative for CD risk studies.

The main clinical role of HLA typing comes from its exceptional negative predictive value for CD when HLA-DQ2.5, DQ2.2, and DQ8 are absent (4). We have shown that HLA genotyping substantially improves the accuracy of serological testing for CD and improves overall cost-effectiveness of CD diagnosis when screening for disease at a population level (6). Recent CD diagnostic guidelines also acknowledge a role for HLA typing in identifying symptomatic children at-risk of CD for further work-up (7). As a result, HLA typing has become an important test in the diagnostic work-up of CD and many laboratories now perform this testing.

A variety of approaches are employed for HLA typing, with the "gold standard" based on direct exon detection i.e. PCR-based methods such as sequence-specific priming or hybridization methods. This approach can provide insight into specific alleles if complete genotyping is performed and the high accuracy of results are

considered essential for the clinical care of individuals. However, for large population screening, the cost of testing can be high. A more affordable approach has been reported based on inferring HLA type from the detection of six SNPs (8-10). The SNP-based HLA typing method is a quick and cost-effective option that relies on linkage disequilibrium between the presence of specific polymorphisms and HLA alleles or groups of alleles. It has the potential to detect all heterozygous and homozygous CD risk genotypes involving HLA-DQ2.5, HLA-DQ8, HLA-DQ2.2, and HLA-DQ7 (8-10). The SNP-based approach is ideally suited for large scale HLA typing needs such as in population studies and is regarded to be of high accuracy, with an error rate of 0.07% to 0.5% (8).

Like many researchers in the field, we employed the SNP-based approach for typing of large populations for CD-associated genotypes. However, a major problem when testing Australian and Mexican populations was a failure to adequately resolve the SNP negatively linked to HLA-DQ2.2 (rs4713586). While the SNP rs4713586 has been useful in Dutch, Spanish, Italian, Finnish, and Hungarian CD and control populations (8, 9), it failed to resolve HLA-DQ2.2 in at least two populations including an Italian study of individuals with potential CD and a Swedish population-wide screening study (11, 12). These two studies opted to remove the negative predicting allele from their analysis and therefore were unable to determine individuals that expressed HLA-DQ2.5 in *trans*. HLA-DQ2.2 is seen in approximately 4% of Europeans with CD (2) and imparts moderate risk for CD

development (4). Therefore, typing strategies need to be able to detect this genotype for a full assessment of CD risk and also to detect genotype combinations such as HLA-DQ8 with HLA-DQ2.2.

Digital droplet PCR enables amplification of both difficult and rare sequences, as the PCR reactions occur in small droplets containing low numbers of template sequences, allowing primers and probes easier access to templates. Here we assess the utility of digital droplet PCR to resolve the challenging rs4713586 SNP in samples requiring definitive determination of HLA-DQ2.2. We show this technology can accurately resolve HLA-DQ2.2 and ensure the full spectrum of CD risk genotypes can be established in large populations.

2. Materials and Methods

2.1 Sample collection

The study was approved by the Human Research Ethics Committee of the Walter and Eliza Hall Institute (no. 09/07). The DNA samples were derived from residual blood from children (n=498) presenting to the emergency department for any condition at the Children's Hospital in the State of Sonora, Mexico. The samples were completely de-identified. Written consent and ethics approval from the Children's Hospital in the State of Sonora was provided for sample collection and testing by our group for the HLA genotypes related to CD.

2.2 HLA SNP typing

SNP typing was performed as previously described using 6 SNPs: rs2187668 (ABI C_58662585_10), rs2395182 (ABI C_11409965_10), rs4713586 (ABI C_27950247_10), rs7775228 (ABI C_29315313_10), rs4639334 (ABI C_42975350_10), and rs7454108 (ABI C_29817179_10), all purchased from Applied Biosystems (8-10). Rs4713586 detects HLA-DQ4 and is a negative predicting allele for HLA-DQ2.2, whereas the others positively predict HLA-DQ2.5, HLA-DQ8, HLA-DQ7, or HLA-DQ2.2. 10ng of DNA was added to 6 wells of a 384-well PCR plate per sample and left to dry overnight covered. Two 'no template' controls wells were included for each SNP. The next day a Master Mix was prepared for each probe pair so that each reaction contained 1x Taqman Universal Master Mix (No AmpErase UNG; provided as 2x) and 1x SNP genotyping assay (provided as 20x) in a final reaction volume of 5µl. Master mix was added to one well for each sample and SNP, and the two no template control wells. The plates were sealed with optical film and centrifuged for 5min at RT at 2000rpm. Allelic discrimination was run on either Applied Biosystems 7900HT or Viia7 instruments. Cycling conditions were as follows: Hold 95^{o}C 10min, followed by 50 cycles of denaturing 92^{o}C 15secs and anneal/extend 60^{o}C 90secs. Pre and post reads were performed. Results were analysed with Applied Biosystems Sequence Detection software. VIC and FAM signals were used to determine genotype calls (8-10).

2.3 Digital droplet PCR

In order to prepare the droplets for the digital PCR, 20ng DNA was mixed with a final concentration of 1x Biorad's digital droplet PCR (no dUTP) Supermix and 1x SNP assay in a final volume of 22µl (excess prepared for droplet generator). 20µl of the PCR mix was added using a multi-channel pipette into one 8 well cartridge in the designated cartridge holder. 70µl droplet generator oil was added to each well and the cartridge was covered with the gasket. Droplets were generated in Biorad's QX100 droplet generator. 40µl droplets were then transferred carefully into a 96-well PCR plate. Alternatively, droplets were generated using the Biorad droplet generator robot. Each droplet contains small amounts of DNA template, probes, and PCR mix. This allows rarer targets to be amplified, as primers have easier access to the DNA templates. The PCR plate was covered in foil and heat sealed. The plate was run on a Biorad thermocycler. Cycling conditions were as follows: Hold 95^{o}C 10 mins, followed by 40 cycles of denaturing 94^{o}C 30secs and anneal/extend 60^{o}C 60 secs, followed by a 2nd hold 98^{o}C for 10mins. Samples were analysed in the Biorad droplet reader with the Biorad QuantaSoft Software.

2.4 HLA typing correlation

A selection of 24 Australian CD patient DNA samples and 29 Mexican DNA samples from the main cohort were genotyped by the Victorian Transplantation and Immunogenetics Service (VTIS) at the Australian Red Cross Blood Service or Melbourne Pathology. Typing to confirm the presence or absence of a CD risk

genotype was performed by either sequence-based typing or microbead hybridisation (9).

3. Results

3.1 Incomplete CD HLA typing using an established six SNP assay

We followed an established HLA SNP typing protocol to determine the presence of HLA-DQ2.5, HLA-DQ8, or HLA-DQ2.2 in 498 DNA samples (8). Results were obtained for 5 out of 6 SNPs. However, for SNP rs4713586 the genotype could not be called due to a lack of clear amplification of the FAM signal compared with the other SNP assays that clearly showed VIC and FAM signals (Figure 1A-C). Although it is possible to obtain information for most of the CD risk genotypes with 5/6 SNPs, any samples called as HLA-DQ2.2 without the rs4713586 SNP may instead be positive for HLA-DQ4 due to its additional tagging by rs2395182 and rs7775228. Therefore, inclusion of rs4713586 allows a positive call for HLA-DQ4, thereby excluding HLA-DQ2.2. To address this shortcoming, we attempted to optimise the assay using DNA samples with known genotype as positive controls. Adjustments to numerous variables including higher concentration of probes, higher DNA amounts, increased reaction volume, and a replacement SNP assay were assessed but none resulted in any changes in FAM signal (data not shown). Increasing the number of PCR cycles from 50 to 60 cycles increased the background FAM signal in known FAM negative samples (data not shown). The forward primer of the rs4713586 assay overlaps four neighbouring SNPs with unknown allele frequency information and it is conceivable

that one or more of these SNPs interfere with the assay. The target sequence contains largely repetitive regions which led us to believe that assay re-design was unlikely to work.

3.2 Digital droplet PCR resolves SNP rs4713586

As expected, most DNA samples did not require resolution of SNP rs4713586 as the combination of the other five SNP results imputed an unequivocal risk genotype i.e. heterozygous or homozygous versions of HLA-DQ2.5, -DQ8 or -DQ7). In addition, samples that were FAM+ for Rs7775228 could be deemed negative for HLA-DQ2.2 and the negative predictive SNP (DQ4) was not required. To assess whether digital droplet PCR could help resolve the rs4713586 SNP, we tested a set of previously HLA typed positive control DNA samples that were positive or negative for rs4713586. We found the digital droplet PCR enabled correct detection of both the FAM and VIC signals in these samples for the rs4713586 allelic discrimination assay (Figure 2).

We then went on to complete the genotyping of the 498 DNA samples, of which 180 (36.2%) required typing of rs4713586. For these 180 DNA samples, the initial five SNP assay results were combined with the rs4713586 digital droplet PCR results in a "two-step" combination. Of these 498 Mexican DNA samples, 152 (30.5%) were HLA-DQ2+, 161 (32.3%) HLA-DQ8+DQ2.5-, 104 (20.9%) either HLA-DQ2.2+ or HLA-DQ7+ (and DQ2.5-DQ8-), and 81 (16.3%) expressed non-CD risk haplotypes. Out of the n=180 DNA samples requiring SNP rs4713586, 12 carried the combination

of HLA-DQ2.2 and DQ7 and therefore expressed the HLA-DQ2.5 heterodimer in *trans*. Determining HLA-DQ2.2 expression required the combination of all three SNPs (rs4713586, rs 2395182, and rs7775228), as discrepancies occurred between rs2395182 and rs7775228. The heterozygous or homozygous status for HLA-DQ2.2 matched with rs7775228.

3.3 SNP assay HLA genotypes correlated with accredited pathology lab results

To confirm the results of the combined SNP and digital droplet PCR approach, a subset of DNA samples (n=53; 24 Australian and 29 Mexican) were genotyped using direct exon detection at one of two commercial labs (Table 1). There was good correlation between the SNP assay typing results and those of the diagnostic labs, with 3 of 53 (5.7%) samples incorrectly called by the SNP assay (Table 1). Two of these (one Mexican and one Australian) occurred in people who expressed DQB1*06:01, which therefore may be detected by rs7775228 in addition to DQB1*02:02, potentially causing the incorrect call. The Mexican sample was also positive for HLA-DQ7, resulting in an incorrect HLA-DQ2.5 in *trans* call. rs7775228 cross-recognition of DQB1*06:01 and DQB1*02:02 is unlikely to cause a major typing issue at a general population level in this study as the frequency of DQB1*06:01 is low (1.3% in Australia and 1.1% in Mexico (13)). The third incorrect call related to HLA-DQ2.5 zygosity status - homozygous on SNP typing compared to heterozygous based on direct exon detection (DQB1 *02:01, 03:01; DQA1 *05:01, 05:03/07). Despite discrepancies between rs2395182 and rs7775228 HLA-DQ2.2

calls, the rs7775228 assay correlated well with results obtained by conventional methods and therefore superseded results for rs2395182. Taken together, this combined two-step typing approach correlates closely with traditional exon detection approaches, but false calls may occur in a small proportion of samples when studying large populations.

3.4 SNP-based CD risk HLA genotype frequencies in the Mexican cohort highlights similarities and differences to European control groups

As specific HLA types contribute to CD risk, we compared the HLA frequencies in our unselected Mexican cohort to those obtained in four published European control cohorts (Table 2) (9). The notable finding was that 63% of the Mexican samples expressed CD risk genotypes putting them potentially at-risk of CD. Only 37% had genotypes not related to CD, compared to 55-68% of European cohorts. Further, the proportion of HLA-DQ8 was higher in the Mexican cohort consistent with previous reports (14-16). To our knowledge, full CD-risk HLA typing in a Mexican population has not been described to this level and the findings suggest that genetic susceptibility for CD conferred by the main HLA genotypes is very high in Mexico. Emerging data suggests the seroprevalence of CD in Mexico is higher than previously recognised, and taken together with our findings, underscores the need for definitive CD prevalence studies (17).

4. Discussion

Screening for the CD HLA risk genotypes using SNP typing has proven to be a cost-effective method that is particularly suitable for large or high-throughput population studies. Here we have shown that in order to obtain full genotyping data the digital droplet PCR system is a useful add-on to help resolve the negative predicting allele rs4713586.

Although the utility of the rs4713586 SNP is variable between researchers, it can be effectively resolved using digital droplet PCR. One explanation for how it may achieve this is the presence of sequence polymorphism in the TaqMan probe binding sites that result in low copy numbers of cross-hybridising PCR products. It is these that can be detected by the increased sensitivity of the digital droplet PCR approach. In our validation study, we found good correlation between the combined and traditional typing methods. We showed two SNPs also may detect other low frequency HLA alleles; rs7775228 (DQB1*06:01) and rs2187668 (DQA1*05:03/07) that could lead to incorrect calls, but this appeared to be uncommon in both the Mexican and Australian samples. Future validation in other populations is important as the presence of different, rare haplotypes may affect the number of false calls.

Although the digital droplet PCR is slightly higher in cost than a standard PCR reaction due to the larger reaction volume, not all samples require the rs4713586 SNP to be resolved. For instance, samples that have been shown homozygous for the DQ2.5, DQ8, or DQ7 SNPs or heterozygous for a combination of these cannot carry DQ2.2. In addition, samples that are negative for the positive predicting DQ2.2 SNPs

do not require ruling out of DQ4. For researchers, it is likely to be significantly more cost-effective to undertake in-house SNP-based typing combined with digital droplet PCR to achieve full genotyping coverage when assessing large populations than utilising a direct exon detection method through a pathology laboratory. While the potential for researchers is substantial, we believe the lack of full sequencing information in the HLA genes and known linkages to the described SNPs currently limit the suitability of this approach for typing of individuals for CD diagnostic purposes. Finally, assessing a broader panel of SNPs may be useful in instances where detection of HLA alleles associated with other autoimmune diseases is sought.

In conclusion, digital droplet PCR aids detection of HLA-DQ2.2 and may be a useful method to amplify and detect other difficult or rare PCR targets. In combination with SNP typing, this is a cost-effective and accurate approach for cohort screening and population typing studies. Further characterisation of the genome regions in linkage disequilibrium with CD-susceptible genotypes will see improvements in accuracy that may eventually support utility in the clinic.

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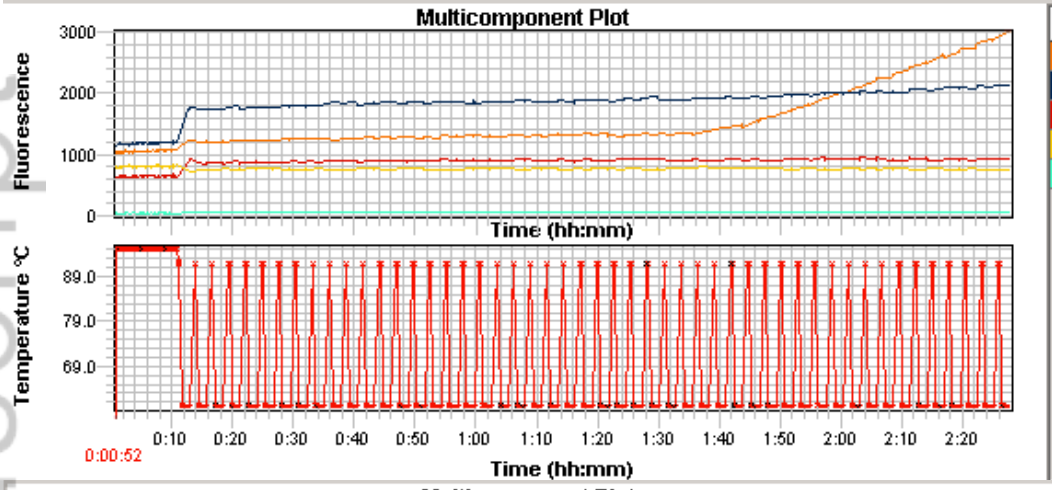
Figure legends

Figure 1 – Example HLA-DQ2.2 negative predicting SNP assay rs4712586 with low/no FAM amplification by real-time PCR. Detection of VIC and FAM signals in amplified DNA for rs 4712586 (A and B) and rs7454108 (C). Representative

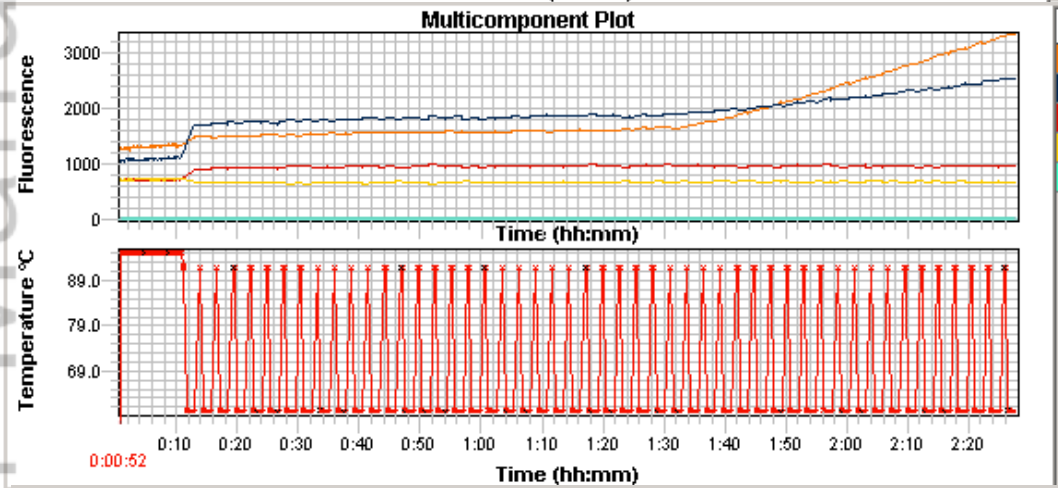
amplification plots of DNA samples with known genotype are shown. **A.** A DNA sample that should amplify as VIC+FAM- is identified correctly on the ABI 7900HT instrument. **B.** A DNA sample that should amplify as VIC+FAM+ is incorrectly identified as VIC+FAM- due to poor FAM amplification. **C.** A DNA sample that should amplify as VIC+FAM+ for one of the 5 working SNPs showing clear distinction of both the VIC and FAM signals.

Figure 2 – Example HLA-DQ2.2 negative predicting SNP assay rs4712586 that is amplified by digital droplet PCR. VIC and FAM signals are detected in amplified DNA for rs4712586 when using digital droplet PCR. **A.** FAM signal from no template control in column A02 and 3 different DNA samples in B02, C02, and D02 showing clear FAM signal in D02. **B.** VIC signals for the same samples in A.

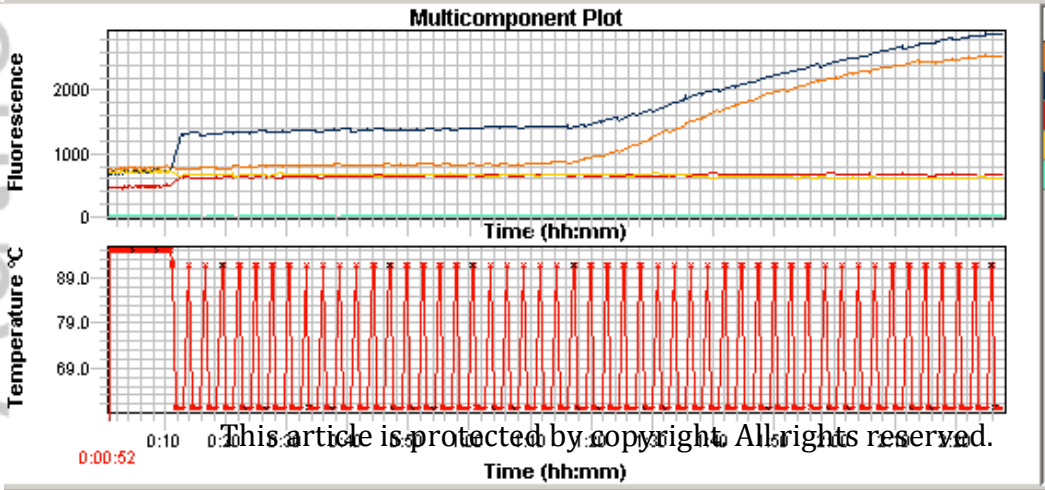
A



B



C



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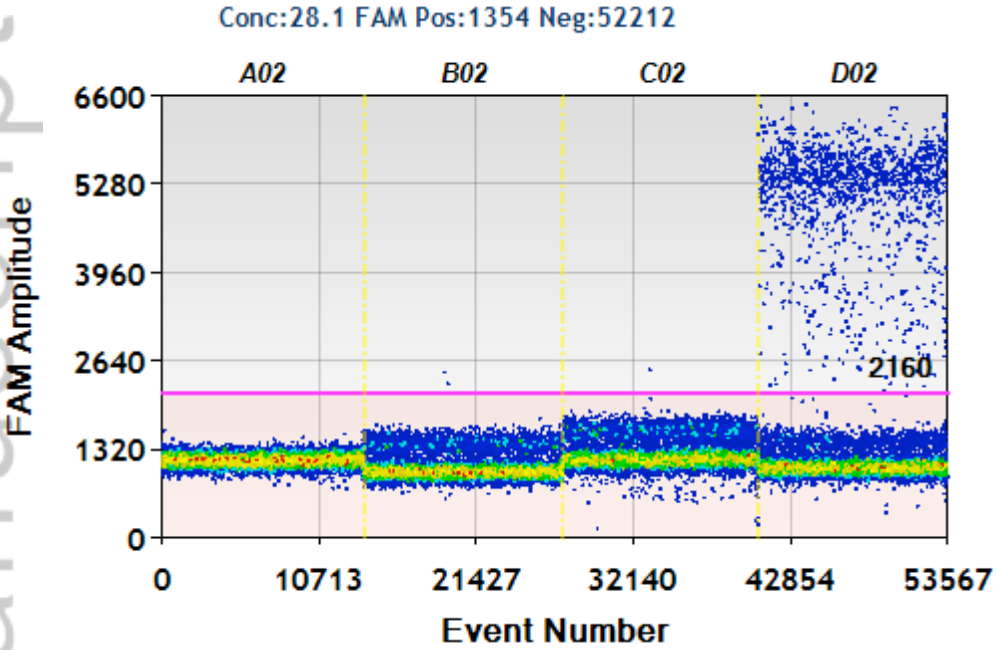

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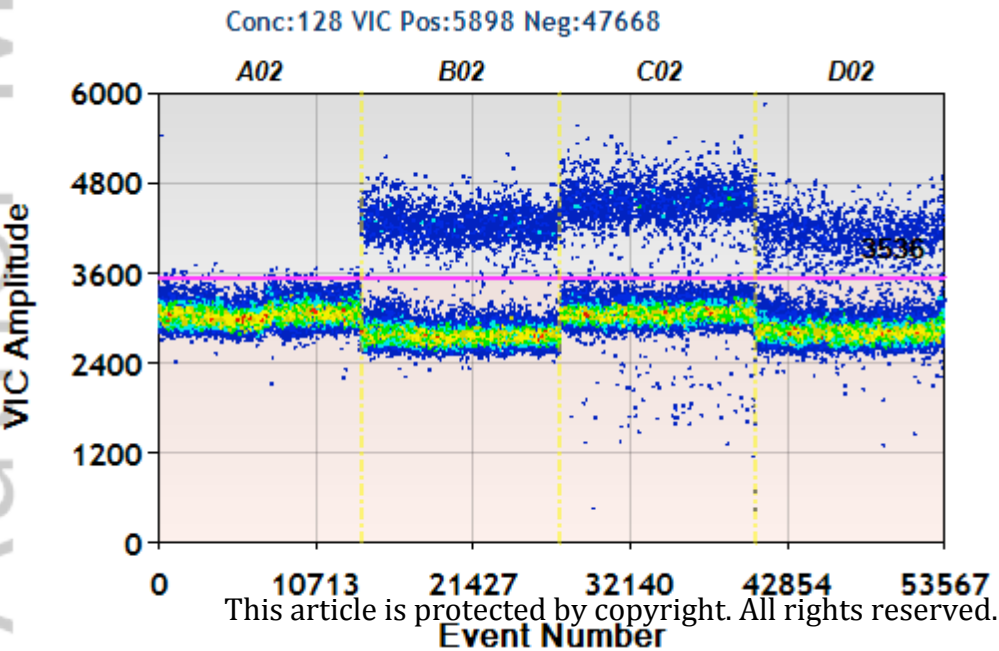

Table 1 – Correlation between SNP assay results and diagnostic lab HLA results

	HLA typing results		Rs 2395182	Rs 4713586	Rs 7775228	Rs 2187668	Rs 79616158	Rs 7454108	
ID	**DQB1**	**DQA1**	**T = DQ2.2+ (T/FAM, G/VIC)**	**G = DQ4+ (or 2.2-; G/FAM, A/VIC)**	**C = DQ2.2+ (T/FAM, C/VIC)**	**T = DQ2.5+ (T/FAM, C/VIC)**	**A = DQ7+ (G/FAM, A/VIC)**	**C = DQ8+ (T/FAM, C/VIC)**	**Genotype**
M1	03:01, 04:02	ND	GT	AG	CT	C	AG	T	DQ7/DQX
M2	03:02, 06:04	01:02, 03:01/02/03	T	A	T	C	G	CT	DQ8/DQX
M3	02:01, 06:02	01:02, 05:01	GT	A	T	CT	G	T	DQ2.5/DQX
M4	02:01, 05:02	01:02, 05:01	T	A	T	CT	G	T	DQ2.5/DQX
M5	03:02	03:01/02/03	T	A	T	C	G	C	DQ8/DQ8
M6	03:01, 03:19	05:05/09	GT	A	T	C	AG	T	DQ7/DQX
M7	03:01, 03:02	03:01, 05:05/09	T	A	T	C	AG	CT	DQ8/DQ7
M8	02:01, 03:02	03:01, 05:01	T	A	T	CT	G	CT	DQ2.5/DQ8
M9	03:01, 05:02	01:02, 05:05/09	T	A	T	C	AG	T	DQ7/DQX
M10	02:01, 04:02	04:01, 05:01	GT	AG	CT	CT	G	T	DQ2.5/DQX
M11	02:01, 02:02	02:01, 05:01	T	A	CT	CT	G	T	DQ2.5/DQ2.2
M12	03:01, 03:02	03:02/03, 05:05/09	T	A	T	C	AG	CT	DQ8/DQ7
M13	02:02, 06:02	01:02, 02:01	GT	A	CT	C	G	T	DQ2.2/DQX
M14	02:01, 03:01	05:01, 05:03/07	T	A	T	T	G	T	DQ2.5/DQ2.5 incorrect
M15	02:01, 03:01	05:01/05/09	T	A	T	CT	AG	T	DQ2.5/DQ7
M16	02:01, 03:01	05:01/05/09	T	A	T	CT	AG	T	DQ2.5/DQ7
M17	02:02, 03:01	02:01, 05:05/09	T	A	CT	C	AG	T	DQ7/DQ2.2
M18	03:01, 05:01	01:01/04/05, 02:01	T	A	T	C	G	T	DQX/DQX
M19	02:01, 05:01	01:01/04/05, 05:01	T	A	T	CT	G	T	DQ2.5/DQX
M20	04:02, 05:01	01:01/04/05, 04:01	T	AG	CT	C	G	T	DQX/DQX
M21	03:01/27/28/29/35, 04:02	04:01, 06:01	T	AG	CT	C	G	T	DQX/DQX
M22	03:03/30/31/33/34, 04:02/04	02:01, 0401	T	AG	CT	C	G	T	DQX/DQX
M23	04:02, 05:01	01:01/04/05, 04:01	T	AG	CT	C	G	T	DQX/DQX
M24	03:01/24/27/28/29/35/36, 06:01/43	01:03, 05:05/09	GT	A	CT	C	AG	T	DQ7/DQ2.2 incorrect
M25	02:02, 03:03/31/33/34	02:01	T	A	CT	C	G	T	DQ2.2/DQX
M26	02:02, 04:02	02:01, 03:01/02/03	T	AG	C	C	G	T	DQ2.2/DQX
M27	02:02, 04:02	02:01, 04:01	GT	AG	C	C	G	T	DQ2.2/DQX
M28	02:02, 05:01	01:01/04/05, 02:01	T	A	CT	C	G	T	DQ2.2/DQX
M29	03:01/19/29, 04:02	ND	T	AG	CT	C	AG	C	DQ7/DQX
A1	02:02, 03:02	02:01, 03:01/02/03	T	A	CT	C	G	CT	DQ8/DQ2.2
A2	02:02, 03:01/04	02:01,	T	A	CT	C	G	T	DQ2.2/DQX

		03:01/02/03							
A3	02:02/14	02:01	T	A	C	C	G	T	DQ2.2/DQ2.2
A4	02:02, 03:02	02, 03:01/02/03	T	A	CT	C	G	CT	DQ8/DQ2.2
A5	02, 03:01/19/21/22	02, 03	T	A	CT	C	G	T	DQ2.2/DQX
A6	05, 06	01, 01	T	A	T	C	G	T	DQX/DQX
A7	03:01/19/21/22, 06	01, 03	GT	A	T	C	G	T	DQX/DQX
A8	02, 02	02, 02	T	A	C	C	G	T	DQ2.2/DQ2.2
A9	02, 06	01, 02	GT	A	CT	C	G	T	DQ2.2/DQX
A10	02, 02	02, 02	T	A	C	C	G	T	DQ2.2/DQ2.2
A11	02, 06	01, 02	GT	A	CT	C	G	T	DQ2.2/DQX
A12	05, 06	01, 01	GT	A	CT	C	G	T	DQ2.2/DQX incorrect
A13	02:01/02/04, 03:01/09/19	02, 05	T	A	CT	C	AG	T	DQ2.2/DQ7
A14	02, 05	01, 02	T	A	CT	C	G	T	DQ2.2/DQX
A15	02, 03:01	02, 05	GT	A	CT	C	AG	T	DQ2.2/DQ7
A16	02:01	05:01/05/09/10/11	T	A	T	T	G	T	DQ2.5/DQ2.5
A17	02:01	05:01/05/09/11	T	A	T	T	G	T	DQ2.5/DQ2.5
A18	02:01	05:01/05/09/11	T	A	T	T	G	T	DQ2.5/DQ2.5
A19	02, 02	05, 05	T	A	T	T	G	T	DQ2.5/DQ2.5
A20	02, 06	01, 05	GT	A	T	CT	G	T	DQ2.5/DQX
A21	02, 02	02, 05	T	A	CT	CT	G	T	DQ2.5/DQ2.2
A22	02, 03:01	05, 05	T	A	T	CT	AG	T	DQ2.5/DQ7
A23	02, 03:03	02, 03	T	A	CT	C	G	T	DQ2.2/DQX
A24	02:01/02/04, 03:02	02, 03	T	A	CT	C	G	CT	DQ8/DQ2.2

X denotes HLA other than HLA-DQ2.5, 2.2, 8, or 7. M = Mexican, A= Australian. ND (not done).

Table 2 – HLA genotype frequencies (%) in the Mexican population compared to published European control populations [9]

Genotype	Mexico (this study) n=498	Finland n= 176	p-value	Hungary n=179	p-value	Italy (Trieste) n=202	p-value	Italy (Milan) n=582	p-value
All DQ2.5+	30.5	17.6	<0.001*	21.8	0.026*	36.1	0.149	22.5	0.003*
DQ2.5/DQX	11.7	13.6	0.694	10.1	0.578	13.4	0.631	6.9	0.007*
DQ2.5/DQ2.5	2.6	0.6	0.103	2.2	0.783	3	0.791	0.7	0.011*
DQ2.5/DQ2.2	3.4	0	0.013*	1.7	0.239	3	0.766	2.9	0.644
DQ2.5/DQ7	3.2	1.1	0.142	3.4	0.928	7.9	0.007*	4.1	0.43
DQ2.5/DQ8	7.2	1.7	0.007*	0.6	<0.001*	1.5	0.003*	1.5	<0.001*
DQ2.2/DQ7	2.4	0.6	0.127	3.9	0.297	7.4	0.002*	6.4	0.002*
All DQ2.5-DQ8+	32.3	19.3	0.001*	17.3	<0.001*	8.9	<0.001*	9.5	<0.001*
DQ8/DQX	16.1	15.9	0.962	10.1	0.05	5	<0.001*	5	<0.001*

DQ8/DQ8	6.2	1.1	0.007*	1.7	0.017*	0	<0.001*	0.3	<0.001*
DQ8/DQ2.2	4.4	1.7	0.102	2.2	0.192	1	0.024*	0.7	<0.001*
DQ8/DQ7	5.6	0.6	0.005*	3.4	0.233	3	0.139	3.4	0.0823
All DQ2.5-DQ8-	37.1	63.1	<0.001*	60.9	<0.001*	55	<0.001*	68	<0.001*
DQ2.2/DQX	7.6	9.7	0.398	10.1	0.312	9.4	0.436	10.3	0.127
DQ2.2/DQ2.2	0.8	0	0.233	0	0.229	1.5	0.411	0.7	0.825
DQ7/DQX	11.8	9.1	0.318	22.3	<0.001*	16.8	0.078	28.4	<0.001*
DQ7/DQ7	0.6	1.1	0.478	5	<0.001*	11.9	<0.001*	6.7	<0.001*
DQX/DQX	16.3	43.2	<0.001*	23.5	0.032*	15.3	0.764	22	0.018*

*Chi-square analysis $p < 0.05$. X denotes HLA other than HLA-DQ2.5, 2.2, 8, or 7.