GRADING PROFICIENCY: WHEN "RIGHT" is "WRONG"

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Introduction

All accredited histocompatibility laboratories are required to participate in proficiency testing (PT). HLA typing PT results are graded based on consensus and scored as good, acceptable, unacceptable, or ungraded. One reason why consensus is not always reached is that different typing platforms (e.g., SSO, RT- PCR, NGS, nanopore sequencing) and reporting strategies (G and P groups vs specific alleles) are employed. This report highlights an instance of DPB1 reporting variability identified when a PT challenge, initially tested by RT-PCR, was re-tested during the validation phase of the Oxford Nanopore (ONT) sequencing platform. Testing by ONT revealed that long-read, whole genome sequencing provides a more accurate assessment of HLA alleles than other testing platforms.

Methods and Materials

The PT challenge sample was from the College of American Pathologists (2024 DML-B, DML-03). Testing was performed on the ONT/GenDx sequencing platform. Results were analyzed with NGSengine from GenDx. Data were further interrogated using the IMGT HLA database (Figure 7) and the HLA-DPB1 allele difference tool (Figure 8) from MLC group.

Results

For this PT challenge, the most common result, reported by 42% of labs, was DPB1*01:01 and 105:01 (Figure 1). This included our laboratory with testing performed by RT PCR. However, the two best matching HLA-DPB1 genotypes from the ONT platform were DPB1*01:01, *665:01 and DPB1*105:01, *1484:01 (Figure 2&3). Based on an intronic difference at position 6271 we were able to rule out the combination DPB1*105:01 and *1484:01 (Figure 5). Importantly, using long-read ONT sequencing we identified differences in Exon 1 at codons -22c and -14b, effectively excluding the combination of DPB1*01:01, *105:01 (Figure 6).

Conclusion

Among 95 reporting labs, our ONT result (DPB1*01:01 and DPB1*665:01) was concordant with 7 labs (7%) receiving an acceptable grade. We speculate that these 7 labs also utilized a long-read sequencing platform as analysis would not be conclusive on other platforms (not confirmed). In this instance, the "acceptable" result of DPB1*105:01 was incorrect. It is interesting to note that 55 labs reported NGS results however only a small number included DPB1*665:01 as an alternative allele indicating NGS platform-based differences. Although the relevance of Exon 1 differences in DPB has not been well studied there could be clinical impact if used for donor and recipient matching. As the HLA field moves steadily towards long-read sequencing as the preferred typing platform, such ambiguities will diminish and eventually disappear.

1st Type	2nd Type	Grade	Freq	%
DPB1				
Higher Resolution				
01:01	105:01	Good	27	28.4
01:01:01	105:01	Good	3	3.2
01:01	Other Allele (665:01)	Acceptable	7	7.4
01:01	Other Allele (105:01)	Acceptable	6	6.3
01:01	Other Allele (105:01:01:02)	Acceptable	1	1.1
01:01	04:02P	Acceptable	13	13.7
01:01P	04:02P	Acceptable	11	11.6
01:01P	105:01	Acceptable	2	2.1
01:01:01G	04:02P	Acceptable	1	1.1
01:01:01G	04:02:01G	Acceptable	10	10.5
01:01:01G	105:01	Acceptable	1	1.1
01:01	04:02:01G	Acceptable	3	3.2
01:01:01	04:02:01G	Acceptable	2	2.1
01:01	<no response given> (but 105:01/665:01/1072:01 not ruled out)	Acceptable	1	1.1
01:01	04:02	Unacceptable	4	4.2
01:01	Other Allele (not specified)	Unacceptable	1	1.1
01:01	04:02G	Unacceptable	1	1.1
01:01/01:01:01	10:01/10:01:01	Unacceptable	1	1.1

Figure 1. CAP participant results.

Locus	Map	Region	LRD	#HP	Noise	ΔSN	ESA	MM	?	IMGT	BMG		
J 2024_DML-03_CAP													9837/15392 (63%)
DPB1	99%	Core+	679	13	5.7%	40%	48%	0		3.56.0	2	C -	DPB1*01:01, DPB1*665:01 [R] 2
		Exon+	676	23	8.0%	38%	48%	0					

Figure 2. DPB1 locus call NGSengine®-Turbo Software

Genotype	Co+	Ex+	In
DPB1*01:01, 665:01	**0**	**0**	**1**
DPB1*105:01, 1484:01	0	0	2

Figure 3. Genotype ranking, NGSengine®-Turbo Software

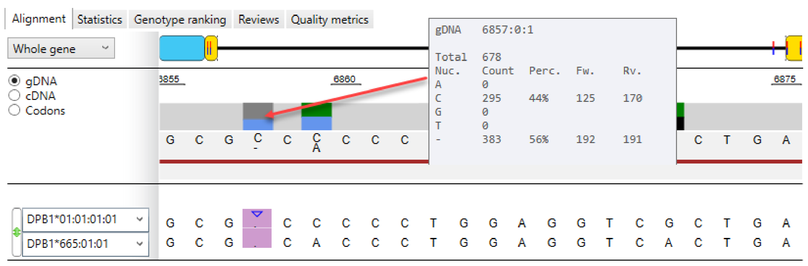

Figure 4. DPB1*665:01 sequence, NGSengine®-Turbo Software

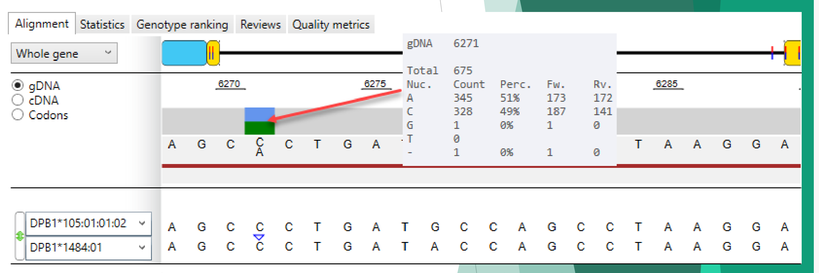

Figure 5. DPB1*105:01 sequence, NGSengine®-Turbo Software.
Note: Presence of "A" eliminates DPB1*105,DPB1*1484:01 combination

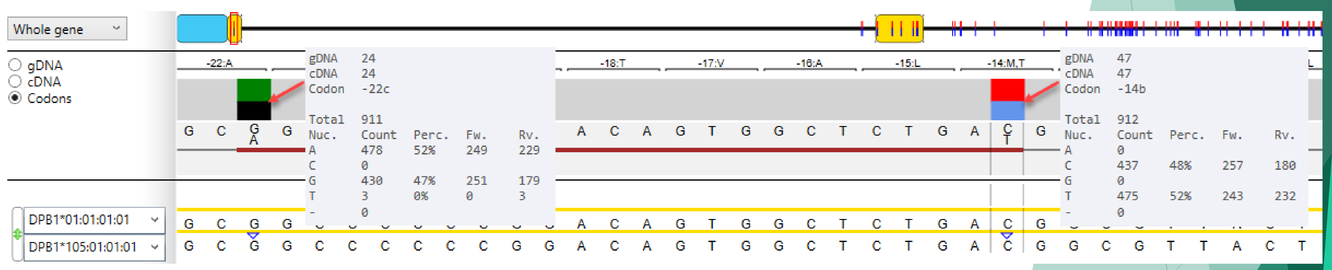

Figure 6. DPB1*01:01, DPB1*105:01 combination, NGSengine®-Turbo Software
Note: Presence of "A" at 24 and "T" at 47 eliminates DPB1*01:01, DPB1*105:01 combination

```
AA Codon                          -25                 -20                 -15                 -10
DPB1*04:02:01:01   ATG ATG GTT CTG CAG GTT TCT GCG GCC CCC CGG ACA GTG GCT CTG ACG GCG TTA CTG ATG
DPB1*105:01:01:01  --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
DPB1*665:01:01     --- --- --- --- --- --- --- --A --- --- --- --- --- --- --- -T- --- --- --- ---
```

Figure 7. IMGT sequence alignment, DPB1 Exon 1
https://www.ebi.ac.uk/ipd/imgt/hla/

HLA-DPB1 Allele Difference Report (source code)

reference allele: HLA-DPB1*04:02:01:01

allele name	SAB	MFI	matches	hypervariable region ID a	b	c	d	e	f	g	protein sequence (aligned to HLA-DPB1*01:01 codons 1-100)
HLA-DPB1*04:02:01:01	Y		7	a3	b1	c3	d1	e2	f2	g2	RATPENYLFQ GRQECYAFNG TQRFLERYIY NREEFVRFDS DVGEFRAVTE LGRPDEEYWN SQKDILEEKR AVPDRMCRHN YELGGPMTLQ RRVQPRVNV
HLA-DPB1*105:01:01:01			7	a3	b1	c3	d1	e2	f2	g2	RATPENYLFQ GRQECYAFNG TQRFLERYIY NREEFVRFDS DVGEFRAVTE LGRPDEEYWN SQKDILEEKR AVPDRMCRHN YELGGPMTLQ RRVQPRVNV
HLA-DPB1*665:01:01			7	a3	b1	c3	d1	e2	f2	g2	RATPENYLFQ GRQECYAFNG TQRFLERYIY NREEFVRFDS DVGEFRAVTE LGRPDEEYWN SQKDILEEKR AVPDRMCRHN YELGGPMTLQ RRVQPRVNV

Figure 8. HLA-DPB1 Allele Difference Report, MLC group
http://apps.mlcgroupllc.com/epitopeFinder/ajaxEpitopeReport.html

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