Omixon Holotype HLA and Omixon HLA Twin

Known product limitations

Version 4

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1 Revision and change history

Version	Summary of changes
v1	Algorithmic limitations collected. Document merged with Holotype HLA-specific limitation document.
v2	Limitations related to the IMGT/HLA database were updated to match IMGT/HLA v3.28.0 and v3.29.0.1. Software limitation section was extended to match the following software versions: Twin 2.1.3, Twin 2.1.4 and Twin 2.5.0.
v3	Additional phasing related cases were added. A short guide added for identifying incorrect phasing. Limitations related to the IMGT/HLA database were updated to match IMGT/HLA v3.30.0. Software limitation section was extended to match the following software versions: Twin 2.5.1 and Twin 3.0.0.
v4	Limitations related to the IMGT/HLA database were updated to match IMGT/HLA v3.31.0. Software limitation section was extended to match the following software versions: Twin 3.1.0 and Twin 3.1.1. Information related to software and IMGT/HLA versions older than 12+1 months was removed. Affected versions: Omixon HLA Twin 2.1.3 and 2.1.4, IMGT/HLA 3.28.0_4. Specific examples were removed for issues where allele specificity could not be proven. Additional limitations were added for the Statistical Genotyping algorithm.

2 The scope of this document

The purpose of this document is to provide a comprehensive list of known product limitations for Holotype HLA and Omixon HLA Twin. The current version (v4) of this document was assembled using Holotype HLA versions 1 and 2.1 and Omixon HLA Twin versions 2.5.0 (CE&RUO), 2.5.1 (CE&RUO), 3.0.0 (RUO), 3.1.0 (RUO), and 3.1.1 (CE&RUO) with IMGT/HLA 3.29.0.1_5, 3.30.0_5, and 3.31.0_5. Unless otherwise specified, the listed limitations are affecting all assay, software and database versions within the scope of this document.

3 Overview of known product limitations

3.1 Holotype HLA-specific limitations

3.1.1 Holotype HLA specific ambiguities

This section contains ambiguities which are caused by the design of the Omixon Holotype HLA assay and technological limitations of NGS (i.e. the location and sequence of primer sites and the fragment size distribution produced by the size selection method used in the protocol). These ambiguities are not resolvable and are presented by all software versions.

A multiple sequence alignment was created for each loci containing all allele sequences and the Holotype primer sequences. Then this alignment was trimmed to the targeted region (i.e. the primer sites and any position outside the primer sites were trimmed). The resulting sequences were than checked for exact duplicates and subsequence relations and all ambiguities on three field or lower resolution or at any resolution but affecting alleles with non-standard expression levels were collected.

3.1.2 First, second and third field ambiguities

Guidelines for Reporting: Report as ambiguous

Ambiguous alleles		Has effect on expression	Affected IMGT/ HLA version(s)	Level of ambiguity	Affected assay version(s)
DPB1*13:01:01	DPB1*107:01	NO	v3.29.0.1_5 v3.30.0_5 v3.31.0_5	1st field	v1, v2.1
DPB1*105:01:01	DPB1*665:01	NO	v3.30.0_5 v3.31.0_5	1st field	v1, v2.1
DQB1*06:01:01	DQB1*06:01:15[1]	NO	v3.29.0.1_5 v3.30.0_5 v3.31.0_5	3rd field	v1
DRB1*09:01:02	DRB1*09:31	NO	v3.29.0.1_5 v3.30.0_5 v3.31.0_5	2nd field	v1, v2.1
DRB1*12:01:01	DRB1*12:10	NO	v3.29.0.1_5 v3.30.0_5 v3.31.0_5	2nd field	v1, v2.1
DRB1*15:02:01	DRB1*15:140	NO	v3.29.0.1_5 v3.30.0_5 v3.31.0_5	2nd field	v1, v2.1

[1]Ambiguity is resolved when DQB1 set 1 primers are used

3.1.3 Ambiguities affecting expression

Guidelines for Reporting: Low-expressing alleles are reported as 2nd field result

Common first three fields in the allele group	4th field in the ambiguous allele group
A*02:01:01	01, *02L*, 16
B*39:01:01	03, *02L*, 05

3.1.4 Cis/Trans ambiguities

Cis/Trans ambiguities (i.e ambiguous allele calls where the different allele pairs only differ in cis/trans phasing) can have multiple root causes. The majority of these ambiguities are reported due to limitations of the technology and the IMGT/HLA database.

3.2 List of known limitations for Omixon HLA Twin

3.3 Known limitations of the Consensus Genotyping Algorithm

3.3.1 Introduction

All limitations listed below were based on observations reported by Holotype HLA customers or made during internal validation and regression testing. Note, that prior to the end of 2018, these observations were made from almost 100,000 samples of Holotype HLA kits sold worldwide.

3.3.2 False novelty called

Infrequently, HLA Twin can report false novelties to the end user. Note, that the vast majority of these false novelties can be eliminated by manual inspection of the results in Omixon HLA Twin by a trained user.

3.3.3 Long novel indels missed

Two cases have been observed, where long novel insertions or deletions have not been reported by Omixon HLA Twin.

3.3.4 Double novel SNP not reported (Fix version: Omixon HLA Twin 2.5.1)

A single case was observed, where two consecutive novel SNPs were not reported.

3.3.5 Incorrect phasing

A low number of cases was observed where the consensus sequences were phased incorrectly.

Identifying incorrectly phased consensus sequences

Incorrect cis/trans phasing can be suspected if one ore more of the following characteristics are observed:

- Two novel alleles are reported within a single best match pair.
- One novel allele and one partially defined allele is reported.
- One or two rare alleles are reported.
- There are several novel positions.

If incorrect phasing is suspected, the user is advised to inspect the results of the statistical genotyping algorithm.

3.3.6 Cis/trans ambiguity due to inefficient phasing

In some rare cases, second or third field level ambiguities are reported due to inefficient phasing. In these cases, reanalysis of the affected loci with more reads is suggested.

3.3.7 Incorrect QC result reported

Failure mode	Fix version	Affected software version(s)
Spot noise ratio values are sometimes assigned to incorrect consensus positions	Twin 3.0.0	Twin 2.5.0, Twin 2.5.1

3.4 Known limitations of the Statistical Genotyping Algorithm

3.4.1 Some exon sequences determined incorrectly in exon-only analyses (Fix version: Omixon HLA Twin 3.1.0)

Failure mode	Fix version	Affected software version
Due to some inconsistencies in the IMGT/HLA database and the IMGT/HLA database handling method introduced in Twin 3.1.0, some region sequences were determined incorrectly for exon-only analyses.	Twin 3.1.0	Twin 3.0.0

4 Known product limitations for HLA-A

4.1 Omixon HLA Twin specific limitations

4.1.1 Known limitations of the Statistical Genotyping algorithm

Known miscalls by the Statistical Genotyping Algorithm

Due to the similarity of the exon sequences of some allele pairs, the statistical genotyping algorithm reports incorrect alleles in some cases for the following allele groups:

- A*24:02/A*24:253

5 Known product limitations for HLA-B

5.1 Holotype HLA-specific limitations

5.1.1 Alleles that may display low amplification

Low amplification means that the generated read count for an allele is not sufficient for genotyping. In extreme cases, the allele might not be reported at all (dropout).

Low amplification alleles	Compensation in HLA Twin	Detection resolution
B*51:01:02	YES	YES

5.2 Omixon HLA Twin specific limitations

5.2.1 Known limitations of the Consensus Genotyping algorithm

Ambiguous result reported due to loss of consensus for one of the alleles

Affected allele	Additionally reported alleles
B*08:01:01	B*08:182, B*08:01:20
B*40:01:02	B*40:01:45
B*35:01:01	B*35:347, B*35:01:23, B*35:42:01

HLA-B*15:01 miscalled

In some rare cases, alleles belonging to the following allele group can be miscalled:

- HLA-B*15:01:01:01,
- HLA-B*15:01:01:02N,

- HLA-B*15:NEW

5.2.2 Known limitations of the Statistical Genotyping algorithm

HLA-B*44:02:01 and HLA-B*44:03:01 are miscalled due to the presence of an identical exon sequence in HLA-C

Statistical genotyping result	Correct result
HLA-B*40:01:02+HLA-B*44:188/HLA-B*44:46	HLA-B*40:01:02+HLA-B*44:03:01
HLA-B*45:01:01+HLA-B*44:188/HLA-B*44:46	HLA-B*45:01:01+HLA-B*44:03:01

6 Known product limitations for HLA-C

6.1 Omixon HLA Twin specific limitations

6.1.1 Known limitations of the Statistical Genotyping Algorithm

Common miscalls by the Statistical Genotyping Algorithm

Due to the similarity of the exon sequences of some allele pairs, the statistical genotyping algorithm reports incorrect alleles in some cases for the following allele groups:

- C*04:01/C*04:09N
- C*07:02/C*07:01/C*07:18

7 Known product limitations for HLA-DPB1

7.1 Holotype HLA-specific limitations

7.1.1 Low or failed amplification for HLA-DPB1 in DP-multiplex

Failure mode	Affected assay version
HLA-DPB1 displays low amplification or fails to amplify	Holotype HLA v1 - 11 locus configuration

7.1.2 Cis/Trans ambiguities

Guidelines for Reporting: It is up to the individual lab whether to report the ambiguity using the G groups or to report the specific allele pairs that are ambiguous.

Ambiguous alleles		Cause of the ambiguity	2-field difference
DPB1*02:01:02+ DPB1*04:02:01	DPB1*105:01+ DPB1*416:01	Lack of phase between exon 2, intron 2 (if applicable) and exon 3	YES
DPB1*03:01:01+ DPB1*04:02:01	DPB1*351:01+ DPB1*463:01	Lack of phase between exons 2 and 3	YES

Ambiguous alleles		Cause of the ambiguity	2-field difference
DPB1*04:01:01+ DPB1*04:02:01	DPB1*105:01 / DPB1*665:01 + DPB1*126:01	Lack of phase between exons 2 and 3	YES
DPB1*04:01:01+ DPB1*13:01:01 (DPB1*107:01)	DPB1*133:01+ DPB1*350:01	Lack of phase between exons 2 and 3	YES
DPB1*04:01:01+ DPB1*14:01:01	DPB1*350:01+ DPB1*651:01	Lack of phase between exons 2 and 3	YES
DPB1*04:02:01+ DPB1*17:01:01	DPB1*105:01+ DPB1*460:01	Lack of phase between exon 2, intron 2 (if applicable) and exon 3	YES
DPB1*04:01:01+ DPB1*463:01	DPB1*105:01+ DPB1*350:01	Lack of phase between exons 2 and 3	YES

7.2 Omixon HLA Twin specific limitations

7.2.1 Known limitations of the consensus genotyping algorithm

Ambiguity not reported

Result called by Twin	Correct result	Affected IMGT/HLA version(s)
DPB1*126:01+DPB1*665:01| DPB1*105:01+DPB1*126:01	DPB1*126:01+DPB1*665:01| DPB1*105:01+DPB1*126:01| **DPB1*04:01+DPB1*04:02**	v3.30.0_5, v3.31.0_5

8 Known product limitations for HLA-DQB1

8.1 Holotype HLA-specific limitations

8.1.1 Alleles that may display low amplification

Low amplification means that the generated read count for an allele is not sufficient for genotyping. In some cases, the allele might not be reported at all (dropout).

Low amplification alleles	Compensation in HLA Twin	Detection resolution
DQB1*03	YES	YES[1]

[1]Suggestion based on Linkage Disequilibrium (LD) with DQA1

9 Known product limitations for HLA-DRB1

9.1 Technological limitations

Moderate allelic imbalance can be observed for alleles with significantly longer sequences than the average (e.g., some HLA-DRB1*04 alleles). In some rare cases, high allelic imbalance can be observed. Sporadically, allele dropouts can be expected.

9.2 Holotype HLA-specific limitations

9.2.1 Non-specific amplification

Failure mode	Possible effects	Affected assay version(s)
In some rare cases, an additional amplicon can be observed in the second half of the gene (from intron 4 throughout the 3'UTR).	If the aspecific amplicon is only present for one of the alleles, false mismatches can be reported for intron 4.	v1

9.2.2 Low amplification

Moderate to high allelic imbalance can be observed for HLA-DRB1*07 alleles in some cases. Very rarely, allele dropouts can be expected.

9.3 Omixon HLA Twin specific limitations

9.3.1 Known limitations of the consensus genotyping algorithm

HLA-DRB1*12:01 ambiguity missed

Result called by Twin	Correct result	Affected IMGT/HLA version(s)
DRB1*12:10	DRB1*12:10/**DRB1*12:01:01**	v3.29.0.1_5, v3.30.0_5, v3.31.0_5

9.3.2 Known limitations of the Statistical Genotyping Algorithm

Common miscalls by the Statistical Genotyping Algorithm

Due to the similarity of the exon sequences of some allele pairs, the statistical genotyping algorithm reports incorrect alleles or does not report inherent ambiguities in some cases for the following allele groups:

- DRB1*08:01:01/DRB1*08:77
- DRB1*09:01:02/DRB1*09:31/DRB1*09:21
- DRB1*15:02:01/DRB1*15:140

10 Known product limitations for HLA-DRB3

10.1 Holotype HLA-specific limitations

10.1.1 Non-specific amplification

Failure mode	Possible effects	Affected assay version(s)
In some rare cases, an additional amplicon can be observed in the second half of the gene (from intron 4 throughout the 3'UTR).	If the aspecific amplicon is only present for one of the alleles, false mismatches can be reported for intron 4.	v1

11 Known product limitations for HLA-DRB4

11.1 Holotype HLA-specific limitations

11.1.1 Alleles that may display low amplification

Low amplification means that the generated read count for an allele is not sufficient for genotyping. In extreme cases, the allele might not be reported at all (dropout). Low amplification and allele dropouts have frequently been observed for HLA-DRB4*01:01. In rare cases, allele dropouts have been reported for HLA-DRB4*01:03 alleles. In both cases, the presence of the allele is suggested based on linkage disequilibrium by Omixon HLA Twin.

11.1.2 Other assay related limitations

False positive concentration measurements for HLA-DRB4

High amplicon concentrations can be observed in some samples even though:

- the individual does not have a copy of the HLA-DRB4 gene or
- the individual does have one or two copies of the HLA-DRB4 gene, but amplification was not successful.

11.2 Omixon HLA Twin specific limitations

11.2.1 Known limitations of the consensus genotyping algorithm

Ambiguity is not reported

Result called by Twin	Correct result
DRB4*01:02N	DRB4*01:02N/DRB4*01:03N
DRB4*01:01:01:01	DRB4*01:01:01:01/DRB4*01:03N