



Omixon Holotype HLA and Omixon HLA Twin

Known product limitations

Version 7

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1 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 of this document was assembled using Holotype HLA versions 1, 2.1, and 3.0 and Omixon HLA Twin versions 2.5.1 (CE&RUO), 3.0.0 (RUO), 3.1.0 (RUO), 3.1.1 (CE&RUO) and 3.1.3 (RUO) with IMGT/HLA versions 3.32.0_5, 3.32.0_7, 3.33.0_7, 3.34.0_8 and 3.35.0_8. Unless otherwise specified, the listed limitations are affecting all assay, software and database versions within the scope of this document.

For an overview of previous versions and changes of this document see the "Revision and change history" section.

2 Overview of known product limitations

2.1 Holotype HLA-specific limitations

2.1.1 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 then 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.

2.1.2 First, second and third field ambiguities

Guidelines for Reporting: Report as ambiguous

Ambiguities affecting all Holotype HLA versions

Ambiguous alleles		Affected IMGT/HLA version(s)	Level of ambiguity
DPB1*13:01:01	DPB1*107:01	All ¹	1st field
DPB1*105:01:01	DPB1*665:01	All ¹	1st field
DPB1*584:01:01	DPB1*584:01:02	v3.35.0_8	3rd field
DRB1*03:01:01	DRB1*03:147	v3.34.0_8 v3.35.0_8	2nd field
DRB1*09:01:02	DRB1*09:31	All ¹	2nd field
DRB1*09:21	DRB1*09:31	All ¹	2nd field
DRB1*12:01:01	DRB1*12:10	All ¹	2nd field
DRB1*15:02:01	DRB1*15:140/ DRB1*15:149	All ¹	2nd field/ 2nd field



¹ All: All database versions within the scope of this document are affected.

Ambiguities affecting all Holotype HLA v1 only

These ambiguities can be resolved when DQB1 set 1 primers are used.

Ambiguous alleles		Affected IMGT/HLA version(s)	Level of ambiguity
DQB1*02:02:01	DQB1*02:02:06	v3.34.0_8 v3.35.0_8	3rd field
DQB1*03:01:01	DQB1*03:297/ DQB1*03:01:41	v3.33.0_7 v3.34.0_8 v3.35.0_8	2nd field/ 3rd field
DQB1*03:01:01	DQB1*03:01:43	v3.35.0_8	3rd field
DQB1*03:02:01	DQB1*03:289	v3.34.0_8 v3.35.0_8	2nd field
DQB1*06:01:01	DQB1*06:01:15	All ¹	3rd field

¹ All: All database versions within the scope of this document are affected.

2.1.3 Ambiguities affecting expression

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

Ambiguous allele groups

- HLA-A*02:01:01:01/**02:01:01:02L**/02:01:01:16/02:01:01:50
- HLA-B*39:01:01:03/**39:01:01:02L**/39:01:01:05/39:01:01:09
- HLA-DQB1*03:01:01:01/03:01:01:10/03:01:01:20/03:01:01:41/03:01:01:43/**03:276N**¹

¹ Ambiguity is present with IMGT/HLA 3.32.0_5 assay versions v2 and v3, but does not affect IMGT/HLA databases v3.33.0 and above. Holotype HLA v1 is affected with all database versions above 3.31.0.

2.1.4 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.

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.

2.2 List of known limitations for Omixon HLA Twin

2.2.1 Known limitations of the Consensus Genotyping Algorithm

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.



False novelty called

Rarely, 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.

2.2.2 Ambiguity is not reported for novel alleles

By design, only a single novel allele is reported by the Consensus Genotyping algorithm. In very rare cases, multiple equally probable novel alleles can be identified, but only one of these options is called by the algorithm.

Novel indels missed

Extremely rarely, novel insertions or deletions are not reported by Omixon HLA Twin.

Incorrect phasing

In rare cases, the consensus sequences is 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.

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.

Incorrect QC result reported

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

2.2.3 No result reported despite successful consensus generation (Fix version: Omixon HLA Twin 3.1.1)

Failure mode	Fix version	Affected software version(s)
In some rare cases, no allele call is reported even though a consensus sequence was successfully generated	Twin 3.1.1	Twin 2.5.1 Twin 3.0.0 Twin 3.1.0



2.2.4 Known limitations of the Statistical Genotyping Algorithm

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

3 Known product limitations for HLA-A

3.1 Omixon HLA Twin specific limitations

3.1.1 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

4 Known product limitations for HLA-B

4.1 Holotype HLA-specific limitations

4.1.1 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

4.2 Omixon HLA Twin specific limitations

4.2.1 Known limitations of the Consensus Genotyping algorithm

Incorrect consensus sequence due to inefficient cross-mapping detection

- In some rare cases ambiguous results are reported due to partial loss of consensus at the consensus start.
- Rarely, false novelties are reported due to an incorrect consensus sequence near the consensus start.

HLA-B*15:01 miscalled

In some rare cases, alleles belonging to the following allele group can be miscalled and amino-acid information can be incorrect due to inconsistencies in the database:



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

4.2.2 Known limitations of the Statistical Genotyping algorithm

Some HLA-B alleles are miscalled due to the presence of an identical exon sequence in HLA-C

A group of HLA-B alleles (several HLA-B*44 alleles and HLA-B*47:04) have an exon 2 sequence identical to HLA-C*16:85. Due to this similarity, these alleles can be miscalled by the statistical genotyping algorithm.

5 Known product limitations for HLA-C

5.1 Omixon HLA Twin specific limitations

5.1.1 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

6 Known product limitations for HLA-DPB1

6.1 Holotype HLA-specific limitations

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

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

6.2 Omixon HLA Twin specific limitations

6.2.1 Known limitations of the consensus genotyping algorithm

Ambiguity not reported

In some rare cases, G-group level cis/trans ambiguities are not reported.



7 Known product limitations for HLA-DQB1

7.1 Holotype HLA-specific limitations

7.1.1 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

¹Suggestion based on Linkage Disequilibrium (LD) with DQA1

7.1.2 Alleles that are not amplified

DQB1*03:276N – due to a long deletion covering the 5' amplification primer site this allele is not amplified.

7.1.3 Low amplification for Holotype HLA v1 set 1 primers

In some cases, the amplicon produced by the Holotype HLA v1 set 1 primers displays low amplification.

8 Known product limitations for HLA-DRB1

8.1 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.

8.2 Holotype HLA-specific limitations

8.2.1 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

8.2.2 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.

8.3 Omixon HLA Twin specific limitations



8.3.1 Known limitations of the consensus genotyping algorithm

HLA-DRB1*12:01 ambiguity is missed in some cases

Result called by Twin	Correct result
DRB1*12:10	DRB1*12:10/ DRB1*12:01:01

8.3.2 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*04:07:01/DRB1*04:92
- DRB1*08:01:01/DRB1*08:77
- DRB1*09:01:02/DRB1*09:31/DRB1*09:21
- DRB1*15:02:01/DRB1*15:140

9 Known product limitations for HLA-DRB3

9.1 Holotype HLA-specific limitations

9.1.1 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

10 Known product limitations for HLA-DRB4

10.1 Holotype HLA-specific limitations

10.1.1 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.

10.1.2 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.

10.2 Omixon HLA Twin specific limitations

10.2.1 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

11 Revision and change history

Version	Approval date	Author	Summary of changes	Approved by
v1	05-Jul-2017	Krisztina Rigó	Algorithmic limitations collected. Document merged with Holotype HLA-specific limitation document.	Efithymia Melista, Zoltán Simon, Peter Meintjes, Gabriella Adlovits
v2	31-Jan-2018	Krisztina Rigó	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.	Efithymia Melista, Gergely Tölgyesi, Gabriella Adlovits
v3	04-Jul-2018	Krisztina Rigó	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.	Efithymia Melista, Gergely Tölgyesi, Gabriella Adlovits
v4	19-Oct-2018	Krisztina Rigó	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.	Márton Pogány, Gergely Tölgyesi, Gabriella Adlovits
v5	14-Jan-2019	Krisztina Rigó	Limitations related to the IMGT/HLA database were updated to match IMGT/HLA v3.32.0. Information related to IMGT/HLA versions older than 12+1 months was removed. Affected version: IMGT/HLA 3.29.0.1_5. The assay limitation sections were extended with the following assay version: Holotype HLA v3.0. Formatting was changed in the "Ambiguities affecting expression" section and a new case was added. An additional case was added to the HLA-DPB1 "Cis/Trans ambiguities" section. Additional minor changes and updates.	Efithymia Melista, Gergely Tölgyesi, Gabriella Adlovits



Version	Approval date	Author	Summary of changes	Approved by
v6	26-Mar-2019	Krisztina Rigó	<p>Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.32.0_7, 3.33.0_7 and 3.34.0_8.</p> <p>Software limitation section was extended to match the following software version: Twin 3.1.3.</p> <p>Information related to software and IMGT/HLA versions older than 12+1 months was removed. Affected versions: Omixon HLA Twin 2.5.0, IMGT/HLA 3.30.0_5 and 3.31.0_5.</p> <p>Product versions affected by the DQB1*03:276N ambiguity have been corrected and updated.</p> <p>Specific examples were removed from the HLA-DPB1 Cis/Trans ambiguities section.</p> <p>Additional minor changes and updates.</p>	Efithymia Melista, Gergely Tölgyesi, Gabriella Adlovits
v7	26-Apr-2019	Krisztina Rigó	<p>Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.35.0_8.</p> <p>A limitation of the statistical genotyping algorithm caused by identical region sequences in different loci has been updated.</p> <p>A limitation of the consensus genotyping algorithm related to novel indels has been updated.</p> <p>Section "First, second and third field ambiguities" was restructured.</p>	Efithymia Melista, Gergely Tölgyesi, Gabriella Adlovits