Holotype HLA RUO and Omixon HLA Twin RUO

Known product limitations

Version 30

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RUO

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 the following:

- Holotype HLA protocol versions 3.0 (RUO), 3.0.1 (RUO) and
- Omixon HLA Twin version 4.8.0 (RUO), 4.8.1 (RUO) and 4.9.0 (RUO) with
- IMGT/HLA versions 3.52.0_9, and 3.53.0_10.

Unless otherwise specified, the listed limitations affect 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 generic product limitations

2.1 Holotype HLA-specific limitations

2.1.1 False-positive results affecting DRB3/DRB4 or DRB5

False-positive results may be observed on very rare occasions for HLA-DRB3, HLA-DRB4, or HLA-DRB5 with Omixon Holotype HLA.

2.1.2 Holotype HLA assay protocol version 3.0 specific limitation

When using the Holotype HLA v3.0 protocol some users may experience the presence of a white precipitate after the adaptor ligation step. We have found that this precipitate is formed by a cross-reaction of a compound in the Promega LR-PCR mix and one in the End Repair buffer. In some cases, this precipitate may have small effects in the final library, but it does not affect the genotyping results. For advice on how to handle this phenomenon please contact support@omixon.com[1] or your Field Application Scientist directly.

2.1.3 Holotype HLA-specific ambiguities

This section contains ambiguities that 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).

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 fields or lower resolution or at any resolution but affecting alleles with non-standard expression levels were collected.

1 mailto:support@omixon.com

2.1.4 First, second, and third field ambiguities

Guidelines for Reporting: Report as ambiguous

Ambiguous alleles		Affected IMGT/HLA version(s)	Level of ambiguity
*DPB1*01:01:01*	*DPB1*1484:01*	All*	1st field
DPB1*02:01:02	DPB1*1315:01/ DPB1*02:01:64	All*	1st field/ 3rd field
*DPB1*04:01:01*	*DPB1*1300:01/* *DPB1*1321:01/* *DPB1*1322:01/* *DPB1*04:01:63/* *DPB1*1436:01/* *DPB1*1444:01Q*	All*/ All*/ All*/ All*/ All*/ All*	1st field/ 1st field/ 1st field/ 3rd field/ 1st field/ 1st field
DPB1*04:02:01	DPB1*1346:01	All*	1st field
DPB1*05:01:01	DPB1*1273:01/ DPB1*05:01:16	All*	1st field/ 3rd field
*DPB1*13:01:01*	*DPB1*107:01*	All*	1st field
DPB1*15:01:01	DPB1*1499:01	v3.53.0_10	1st field
*DPB1*39:01:01*	*DPB1*39:01:02*	All*	3rd field
*DPB1*105:01:01*	*DPB1*665:01:01/* *DPB1*1072:01*	All*	1st field
DPB1*296:01	DPB1*1286:01	All*	1st field
DPB1*584:01:01	DPB1*584:01:02	All*	3rd field
DRB1*01:01:01	DRB1*01:100/ DRB1*01:01:35/ DRB1*01:01:41/ DRV1*01:144	All*/ All*/ All*/ v3.53.0_10	2nd field/ 3rd field/ 3rd field
*DRB1*03:01:01*	DRB1*03:147/ *DRB1*03:01:31*	All*	2nd field/ 3rd field
DRB1*04:04:01	DRB1*04:365	All*	2nd field

Ambiguous alleles		Affected IMGT/HLA version(s)	Level of ambiguity
DRB1*04:06:02	DRB1*04:354	All*	2nd field
DRB1*07:01:01	DRB1*07:139/ DRB1*07:151	All*/ v3.53.0_10	2nd field
*DRB1*08:01:01*	*DRB1*08:105*	All*	2nd field
DRB1*09:01:02	DRB1*09:31:02	All*	2nd field
DRB1*10:01:01	DRB1*10:38	All*	2nd field
DRB1*12:01:01	DRB1*12:10	All*	2nd field
DRB1*12:02:01	DRB1*12:101	All*	2nd field
*DRB1*13:01:01*	*DRB1*13:01:34*	All*	3rd field
DRB1*14:25:01	DRB1*14:25:02	All*	3rd field
DRB1*14:54:01	DRB1*14:216/ DRB1*14:243/ DRB1*14:253	All*/ All*/ All*	2nd field
DRB1*15:01:01	DRB1*15:204	All*	2nd field
DRB1*15:02:01	DRB1*15:140/ DRB1*15:149	All*	2nd field
*DRB1*15:03:01*	DRB1*15:185	All*	2nd field
*DRB1*15:07:01*	*DRB1*15:198*	All*	2nd field
DRB1*16:02:01	DRB1*16:64	All*	2nd field
*DRB3*01:01:02*	*DRB3*01:62:01/ DRB3*01:01:16*	All*/ All*	2nd field/ 3rd field

Ambiguous alleles		Affected IMGT/HLA version(s)	Level of ambiguity
DRB3*02:02:01	DRB3*02:144/ DRB3*02:167/ DRB3*02:168/ DRB3*02:188/ DRB3*02:189/ DRB3*02:193/ DRB3*02:02:34/ DRB3*02:02:35	All*/ All*/ All*/ All*/ All*/ v3.53.0_10/ All*/ All*	2nd field/ 2nd field/ 2nd field/ 2nd field/ 2nd field/ 2nd field/ 3rd field/ 3rd field
DRB4*01:01:01	DRB4*01:01:11/ DRB4*01:156/ DRB4*01:168	v3.53.0_10 All*/ All*	3rd field/ 2nd field/ 2nd field
DRB4*01:03:01	DRB4*01:134	All*	2nd field
DRB5*01:01:01	DRB5*01:126	All*	2nd field

*All: All database versions within the scope of this document are affected.
Ambiguities in *italic* should be systematically reported as ambiguous from Omixon HLA Twin 4.9.0 and above (regardless of the presence of intronic mismatches), while with earlier software versions these alleles are excluded in case intronic mismatches are detected.

2.1.5 Ambiguities affecting expression

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

2.1.5.1 Ambiguous allele groups

Commonly reported with all:

- HLA-A*02:01:01:01/**02:01:01:02L**/
  02:01:01:16/02:01:01:50/02:01:01:150/02:01:01:159/02:01:01:206/02:01:01:245
- HLA-B*39:01:01:03/**39:01:01:02L**/39:01:01:05/39:01:01:09/39:01:01:15/39:01:01:16/39:01:01:17/
  39:01:01:23/39:01:01:26/39:01:01:27/39:01:01:28/39:01:01:29
- HLA-DPA1***03:05:01:01Q**/**03:05:01:02Q**
- HLA-DPB1*04:01:01/04:01:63/1321:01/1322:01/1436:01/**1444:01Q**[4]
- HLA-DPB1*1373:01/**1442:01N**[4]

Only reported with Omixon HLA Twin 4.9.0 and above in rare cases:

- HLA-DPB1*01:01:01/01:01:07/417:01:01/1050:01:01/1151:01/**1332:01N**/**1325:01N**[3]/1443:01/1484:01
- HLA-DQB1*03:01/**03:01:01:21N**[2]/03:19/03:29/03:191/**03:276N**/
  03:297/03:309:02/03:312/03:377/03:419/03:431/**03:358N**[1]/03:454/03:45/03:483/03:497/03:508

[1] Ambiguity is present with IMGT/HLA 3.36.0_8 and above. HLA-DQB1*03:358N contains a deletion in exon 3, which causes a frameshift and a premature stop in codon 191 (source: http://hla.alleles.org/alleles/nulls.html, date of

access: 16-Jul-2019). As of 16-Jul-2019, this allele has been observed in two biological samples with unknown ethnic origin by a single laboratory. No information was publicly available about the source sequences for this allele at the time this document was created. Based on the information available in IMGT/HLA 3.36.0, this allele cannot be distinguished from the other alleles listed in the ambiguous allele groups by the software, but it can be ruled out by manual comparison of the allele sequences in the gene browser. Note, that not all listed alleles are reported as ambiguous in all cases.

[2] DQB1*03:01:01:21N was added in IMGT/HLA 3.47.0. Note, that this allele can be distinguished from normally expressed DRB1*03 alleles, but cannot be distinguished from DQB1*03:358N.

[3] The allele was added in IMGT/HLA 3.49.0.

[4] The allele was added in IMGT/HLA 3.51.0.

2.1.6 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 the 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

2.2.1.1 Introduction

All limitations listed below were based on observations reported by Omixon's customers or made during internal validation and regression testing.

2.2.1.2 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.

In some very rare cases, a novel allele is reported, even though an allele with no exonic mismatches is available.

2.2.1.3 Exon mismatch reported instead of novel allele

In very rare cases, a best match with an exonic mismatch is reported instead of a novel allele.

2.2.1.4 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.

2.2.1.5 Long novel indels missed

In some cases, long novel insertions or deletions are not reported by Omixon HLA Twin.

2.2.1.6 Null allele called instead of a novel allele

In some cases, an unambiguous null allele is reported instead of a novel allele. Known cases affect the following alleles (but may not be limited to):

- HLA-DQB1*03:276N/*03:358N/*03:338N called instead of HLA-DQB1*03:01:01:XX novel.

2.2.1.7 Null allele reported as ambiguous alongside normally expressed allele

In some cases, null alleles that differ from normally expressed alleles in deletions are reported as ambiguous alongside the normally expressed allele. Note that these ambiguities can be resolved by manually inspecting the result in the gene browser.

2.2.1.8 Incorrect phasing

In rare cases, the consensus sequences are phased incorrectly.

2.2.1.8.1 Identifying incorrectly phased consensus sequences

Incorrect cis/trans phasing can be suspected if one or 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 are 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.

2.2.1.9 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, a reanalysis of the affected loci with more reads is suggested.

2.2.1.10 Ambiguity not reported (Fixed in Omixon HLA Twin 4.9.0)

By design, the algorithm reports alleles with the lowest number of exonic and intronic mismatches compared to the consensus sequence. For genes with partial coverage, it can rarely happen that the correct allele is only reported as a remainder allele as it has a higher number of intronic mismatches than the best matching allele in the region that is covered by the amplicon.

In some rare cases:

- G-group level cis/trans ambiguities are not always reported for HLA-DPB1.
- The following common ambiguities are not always reported (reported alleles/**not reported alleles**):
  - HLA-DPB1*1072:01/**DPB1*105:01**
  - HLA-DRB1 - DRB1*12:10/**DRB1*12:01:01,** DRB1*15:140/DRB1*15:149/**DRB1*15:02,** DRB1*03:147/**DRB1*03:01:01**, DRB1*14:216/DRB1*14:243**/DRB1*14:54**
  - HLA-DQB1- DQB1*03:276N/**DQB1*03:01**.

2.2.2 Known limitations of the Statistical Genotyping Algorithm

Due to the high similarity of the exon sequences of some alleles, the statistical genotyping algorithm reports incorrect alleles in some cases.

2.2.3 Other limitations

A limitation has been identified in version 4.8.0 of the Twin Server - Client solution. When attempting to open 48 results or more using the "View Result" feature, the Client application may display an Unrecoverable Network Error message. (This issue is fixed in Omixon HLA Twin 4.8.1.)

In some rare cases where the reported alleles have significant length differences in some gene regions, the sequence tracks in the gene browser can be incorrectly aligned, and unnecessary gaps are shown. This issue does not affect the allele call or the values calculated for QC metrics. Displaying alleles separately can eliminate this visualization defect.

Ambiguity reporting on the analysis result screen may not be optimal in cases where alleles are duplicated on both chromosomes due to the ordering of alleles in pairs.

Note that when using the Bulk assignment wizard in the Allele Assignment toolbar and selecting the 'Assign only unambiguous results' option, there is a limitation in cases of DRB3/4/5 genotypes. Ambiguous results may still be assigned due to the homozygous or hemizygous status of these genotypes being based on the DRB1 haplotype, which is not considered in this feature.

For novel alleles, the amino acid track does not function correctly in Omixon HLA Twin versions 4.8.0 and 4.8.1.

3 Known product limitations for HLA-B

3.1 Holotype HLA-specific limitations

3.1.1 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

3.2 Omixon HLA Twin specific limitations

3.2.1 Known limitations of the Consensus Genotyping algorithm

3.2.1.1 Incorrect consensus sequence due to inefficient cross-mapping detection

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

3.2.1.2 HLA-B*15:01 miscalled

In some very 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

3.2.2 Known limitations of the Statistical Genotyping algorithm

3.2.2.1 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.

4 Known product limitations for HLA-DQB1

4.1 Alleles that are not amplified

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

5 Known product limitations for HLA-DRB1

5.1 Technological limitations

Moderate allelic imbalance can be observed for alleles with significantly longer sequences than the average (such as some HLA-DRB1*04 alleles). In some rare cases, a high allelic imbalance can be observed. Very rarely, an allele dropout may occur.

5.1.1 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.

6 Known product limitations for HLA-DRB4

6.1 Holotype HLA-specific limitations

6.1.1 Alleles that are not amplified

DRB4*03:01N – due to a long deletion covering the 5'amplification primer site this allele is not amplified.

6.2 Omixon HLA Twin specific limitations

6.2.1 Known limitations of the consensus genotyping algorithm

6.2.1.1 Ambiguity is not reported

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

7 Revision and change history

Version	Approval date	Summary of changes
v1	05 Jul 2017	Algorithmic limitations collected. Document merged with the Holotype HLA-specific limitation document.
v2	31 Jan 2018	Limitations related to the IMGT/HLA database were updated to match IMGT/HLA v3.28.0 and v3.29.0.1. The 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	04 Jul 2018	Additional phasing-related cases were added. A short guide was added for identifying incorrect phasing. Limitations related to the IMGT/HLA database were updated to match IMGT/HLA v3.30.0. The software limitation section was extended to match the following software versions: Twin 2.5.1 and Twin 3.0.0.
v4	19 Oct 2018	Limitations related to the IMGT/HLA database were updated to match IMGT/HLA v3.31.0. The 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.
v5	14 Jan 2019	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.

Version	Approval date	Summary of changes
v6	26 Mar 2019	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. The software limitation section was extended to match the following software version: Twin 3.1.3. 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. Product versions affected by the DQB1*03:276N ambiguity have been corrected and updated. Specific examples were removed from the HLA-DPB1 Cis/Trans ambiguities section. Additional minor changes and updates.
v7	26 Apr 2019	Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.35.0_8. A limitation of the statistical genotyping algorithm caused by identical region sequences in different loci has been updated. A limitation of the consensus genotyping algorithm related to novel indels has been updated. Section "First, second, and third field ambiguities" was restructured.
v8	19 Jul 2019	Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.36.0_8. Information related to software and IMGT/HLA versions older than 12+1 months was removed. Affected versions: Omixon HLA Twin 2.5.1, IMGT/HLA 3.32.0_5, 3.32.0_7. A limitation of the consensus genotyping algorithm affecting HLA-DRB3 was added.
v9	08 Aug 2019	The software limitation section was extended to match the following software version: Omixon HLA Twin 4.0.0 Information related to software versions older than 12+1 months was removed. Affected version: Omixon HLA Twin 3.0.0. A limitation of the consensus genotyping algorithm affecting HLA-DRB1 was added.
v10	16 Oct 2019	The software limitation section was extended to match the following software version: Omixon HLA Twin 4.0.1.

Version	Approval date	Summary of changes
v11	19 Nov 2019	Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.37.0_8. Information related to software and IMGT/HLA versions older than 12+1 months was removed. Affected versions: Omixon HLA Twin 3.1.0, IMGT/HLA 3.33.0_7. Limitations of the Statistical Genotyping algorithm and ambiguity-related limitations of the Consensus Genotyping algorithm were restructured.
v12	07 Jan 2020	Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.38.0_8. The software limitation section was extended to match the following software version: Twin 4.1.0. Information related to software and IMGT/HLA versions older than 12+1 months was removed. Affected versions: IMGT/HLA 3.34.0_8.
v13	26 Mar 2020	Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.38.0_9. The software limitation section was extended to match the following software version: Twin 4.2.0. Information related to software and IMGT/HLA versions older than 12+1 months was removed. Affected versions: IMGT/HLA 3.35.0_8. A limitation of the consensus genotyping algorithm affecting recent IMGT/HLA versions was added. The assay limitation section was extended with sporadically occurring DRB3/4/5 specific false positive issue, and assay workflow 3.0 specific phenomenon of the "white precipitant" and its suggested handling.
v14	01 Jul 2020	Information related to software and IMGT/HLA versions older than 12+1 months was removed. Affected versions: IMGT/HLA 3.36.0_8, Omixon HLA Twin versions 3.1.1 and 3.1.3. Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.39.0_9. The software limitation section was extended to match the following software version: Twin 4.2.2. Information related to Holotype HLA v1 was removed as this assay version is no longer supported.

Version	Approval date	Summary of changes
v15	07 Aug 2020	The software limitation sections were extended to match the following software version: Twin 4.3.0. Some limitations affecting Twin versions 4.1.0, 4.2.0, and 4.2.2 were added.
v16	05 Oct 2020	Information related to software versions older than 12+1 months was removed. Affected version: Omixon HLA Twin 4.0.0.
v17	14 Jan 2021	Information related to software and IMGT/HLA versions older than 12+1 months was removed. Affected version: Omixon HLA Twin 4.0.1, IMGT/HLA 3.37.0_8, 3.38.0_8, and 3.38.0_9. The software limitation sections were extended to match the following software version: Omixon HLA Twin 4.4.0 (CE&RUO). Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.40.0_9, 3.41.2_9, and 3.42.0_9, including limited version compatibility with Omixon HLA Twin. A new limitation of the consensus genotyping algorithm was added affecting novel alleles where the novel position is present within a deletion in a null allele similar to the novel allele.
v18	02 Mar 2021	Information related to software versions older than 12+1 months was removed. Affected version: Omixon HLA Twin 4.1.0. The software limitation sections were extended to match the following software version: Omixon HLA Twin 4.4.1 (CE&RUO). Two limitations affecting Omixon HLA Twin 4.4.0 were added (automated genotyping is not functional, gene browser throws an error for some novelties).
v19	07 Jun 2021	Information related to software and IMGT/HLA versions older than 12+1 months was removed. Affected versions: Omixon HLA Twin 4.1.0, Omixon HLA Twin 4.2.0, IMGT/HLA 3.39.0_9, and IMGT/HLA 3.40.0_9. Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.43.0_9.

Version	Approval date	Summary of changes
v20	16 Nov 2021	Information related to software and IMGT/HLA versions older than 12+1 months was removed. Affected versions: Omixon HLA Twin 4.2.2, Omixon HLA Twin 4.3.0, IMGT/HLA 3.41.2_9. Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.44.1_9. Information related to Holotype protocol versions 2.1 (RUO&CE) and 2.2 (RUO) was removed from the document as these product versions were discontinued.
v21	12 Jan 2022	Information related to software and IMGT/HLA versions older than 12+1 months was removed. Affected versions: IMGT/HLA 3.42.0_9. Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.45.1_9.
v22	07 Mar 2022	Information related to software and IMGT/HLA versions older than 12+1 months was removed. Affected versions: IMGT/HLA 3.43.0_9 and Omixon HLA Twin 4.4.0 (CE&RUO). The software limitation sections were extended to match the following software version: Omixon HLA Twin 4.6.0 (CE&RUO).
v23	30 Jun 2022	Information related to software and IMGT/HLA versions older than 12+1 months was removed. Affected versions: IMGT/HLA 3.44.1_9 and Omixon HLA Twin 4.4.1 (CE&RUO). Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.46.0_9 and 3.47.0_9. The scope of the document was extended with Holotype HLA protocol version 3.0.2 (CE&RUO).
v24	14 Oct 2022	The scope of the document was extended with Omixon HLA Twin 4.7.0 (RUO).
v25	06 Dec 2022	Minor updates related to document scope (clearer distinction between CE and RUO products). Information about software and IMGT/HLA versions older than 12+1 months was removed. Affected versions: IMGT/HLA 3.45.1_9, IMGT/HLA 3.46.0_9. Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.48.0_9.

Version	Approval date	Summary of changes
v26	20 Mar 2023	Information related to software and IMGT/HLA versions older than 12+1 months was removed. Affected versions: IMGT/HLA 3.47.0_9. Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.49.0_9, IMGT/HLA 3.50.0_9, and IMGT/HLA 3.51.0_9. The scope of the document was extended with Omixon HLA Twin 4.8.0. Minor limitations of the consensus genotyping algorithm and the user interface were added.
v27	13 Jun 2023	Information about software and IMGT/HLA versions older than 12+1 months was removed. Affected versions: IMGT/HLA 3.48.0_9, Omixon HLA Twin 4.6.0 RUO The scope of the document was extended with Omixon HLA Twin 4.8.1.
v28	03 Aug 2023	Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.52.0_9. Some corrections were made in the ambiguity table (DRB1*12:101 was corrected, DRB1*04 ambiguities were added, and other minor corrections were made). Minor changes in the phrasing were made for the following limitations: Incorrect phasing, Ambiguity not reported
v29	22 Nov 2023	Limitations related to the IMGT/HLA database were updated to match IMGT/HLA 3.53.0_10. Information about software and IMGT/HLA versions older than 12+1 months was removed. Affected versions: IMGT/HLA 3.49.0_9 A limitation of the consensus genotyping algorithm (reporting of non-inherent null allele ambiguities) was added. A limitation of the amino acid track affecting novel alleles was added.
v30	27 Mar 2024	Information about software and IMGT/HLA versions older than 12+1 months was removed. Affected versions: IMGT/HLA 3.50.0_9, IMGT/HLA 3.51.0_9 and Omixon HLA Twin 4.7.0 RUO The scope of the document was extended with Omixon HLA Twin 4.9.0.