# TEC533_TRUSIGHT HLA ASSIGN INSERTION ALIGNMENT AMBIGUITIES

Due to a limitation with the TruSight HLA Assign software, alleles that differ within an insertion cannot be resolved. This limitation is likely to become increasingly problematic in parallel with the ongoing rapid accumulation of HLA NGS data.

## Impact and Presentation

Alleles that differ within an insertion will present as an ambiguity. An example of this is C*17:01:01/C*17:43, where TruSight HLA Assign is unable to resolve the insertion in exon 5.

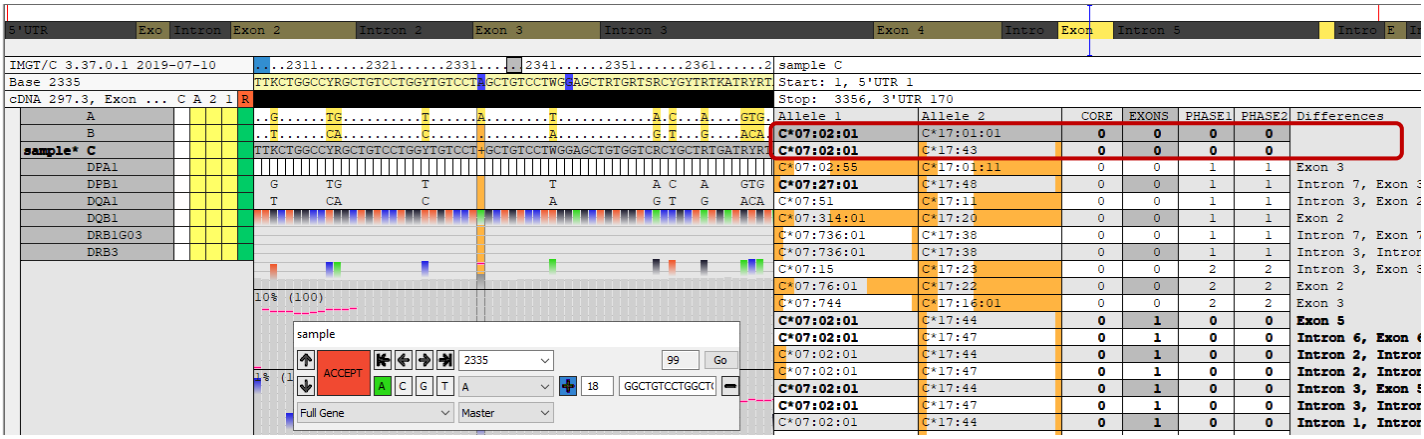


Fig 1. Presentation of C*17:01:01/C*17:43 in TruSight HLA Assign

As C*17:43 differs from C*17:01:01 at position 958, it should be possible to resolve this ambiguity.

| cDNA | 900 | 910 | 920 | 930 | 940 | 950 | 960 | 970 | 980 |
|---|---|---|---|---|---|---|---|---|---|
| C*01:02:01:01 | AGCCG TCTTCCCAGC | CCACCATCCC | CATCGTGGGC | ATCGTTGCTG | GCCTGGCTG..... | .....T | CCTGGCTGTC | CTAGCTGTCC | TAGGAGCTGT |
| C*17:01:01:02 | ----- ---------- | ---------- | --A-T----- | ------T--- | ---CA----TCCTGGCTGT | CCTGGCTG- | ---------- | ---------- | ---------- |
| C*17:01:01:03 | ----- ---------- | ---------- | --A-T----- | ------T--- | ---CA----TCCTGGCTGT | CCTGGCTG- | ---------- | ---------- | ---------- |
| C*17:01:01:04 | ----- ---------- | ---------- | --A-T----- | ------T--- | ---CA----TCCTGGCTGT | CCTGGCTG- | ---------- | ---------- | ---------- |
| C*17:01:01:05 | ----- ---------- | ---------- | --A-T----- | ------T--- | ---CA----TCCTGGCTGT | CCTGGCTG- | ---------- | ---------- | ---------- |
| C*17:01:01:06 | ----- ---------- | ---------- | --A-T----- | ------T--- | ---CA----TCCTGGCTGT | CCTGGCTG- | ---------- | ---------- | ---------- |
| C*17:01:01:07 | ----- ---------- | ---------- | --A-T----- | ------T--- | ---CA----TCCTGGCTGT | CCTGGCTG- | ---------- | ---------- | ---------- |
| C*17:01:01:08 | ----- ---------- | ---------- | --A-T----- | ------T--- | ---CA----TCCTGGCTGT | CCTGGCTG- | ---------- | ---------- | ---------- |
| C*17:01:01:09 | ----- ---------- | ---------- | --A-T----- | ------T--- | ---CA----TCCTGGCTGT | CCTGGCTG- | ---------- | ---------- | ---------- |
| C*17:43 | ----- ---------- | ---------- | --A-T----- | ------T--- | ---CA----TCCTGGCTGT | CCTGGCTG- | -------A-- | ---------- | ---------- |

Fig 2. Alignment of C*17:01:01 and C*17:43 with IMGT v3.38.0.

However, due to differences in the alignment algorithms between TruSight HLA Assign and IMGT, TruSight HLA Assign puts this polymorphism in the insertion. Because of this, this ambiguity cannot be resolved in TruSight HLA Assign.

The alignments differ as TruSight HLA Assign's alignment algorithm sees the sequence GGCTGTCCT(indicated below in pink) as the sequence replicated twice in the C*17:01:01 alleles and inserts the repeats(yellow) 3' of this at position 963, compared to IMGT's replication of TCCTGGCTG(blue) and insertion(yellow) at position 950.

## TruSight HLA Assign:

C*17:01:01 AGCCGTCTTCCCAGCCCACCATCCCCAACTTGGGCATCGTTTCTGGCCCAGCTGTCCTGGCTGTCCTGGCTGTCCTGGCTGTCCTAGCTGTCCTAGGAGCTGTGGTCGCTGCTGTGA

C*17:43 AGCCGTCTTCCCAGCCCACCATCCCCAACTTGGGCATCGTTTCTGGCCCAGCTGTCCTGGCTGTCCTGGCTGTCCTGGCAGTCCTAGCTGTCCTAGGAGCTGTGGTCGCTGCTGTGA

## IMGT:

C*17:01:01 AGCCGTCTTCCCAGCCCACCATCCCCAACTTGGGCATCGTTTCTGGCCCAGCTGTCCTGGCTGTCCTGGCTGTCCTGGCTGTCCTAGCTGTCCTAGGAGCTGTGGTCGCTGCTGTGA

C*17:43 AGCCGTCTTCCCAGCCCACCATCCCCAACTTGGGCATCGTTTCTGGCCCAGCTGTCCTGGCTGTCCTGGCTGTCCTGGCAGTCCTAGCTGTCCTAGGAGCTGTGGTCGCTGCTGTGA

Fig 3. Shaded alignment of C*17:01:01 and C*17:43 indicating the location of the sequences replicated and inserted.

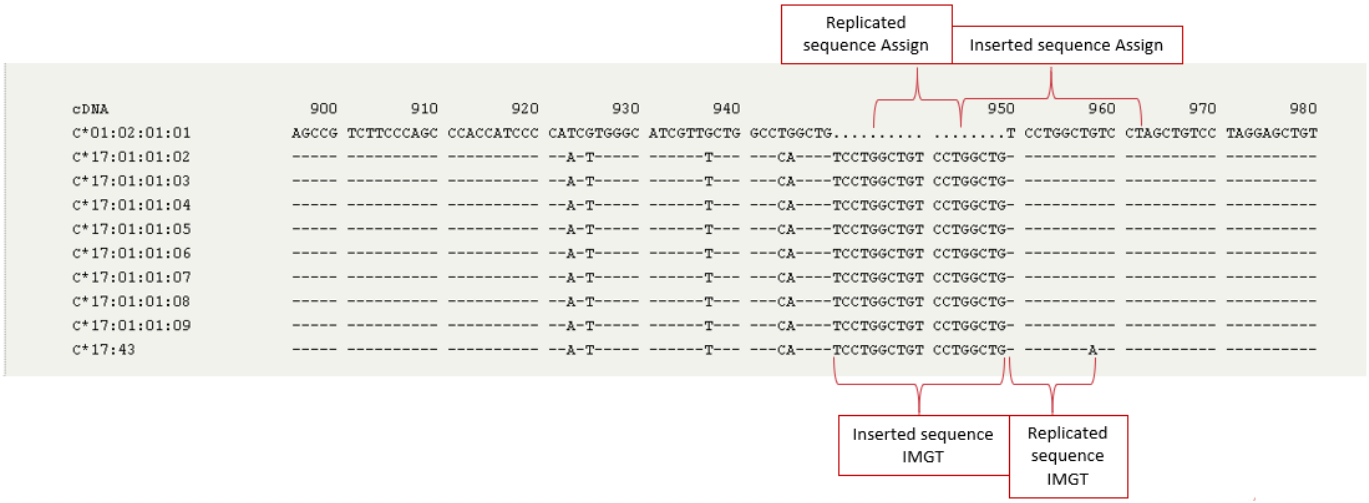


Fig 4. Alignment in IMGT indicating the location of the sequences replicated and inserted.

# Work around

If an ambiguity due to a difference in the insertion is suspected, check the alignment of the ambiguous alleles. Check the location of the insertion in TruSight HLA Assign by viewing the alignment of the reference sequences. To do this select References in the Views section of the Home Ribbon, then enter the names of the alleles to align in the box in the navigator and click filter. Any heterozygous positions and mismatches will be highlighted yellow.

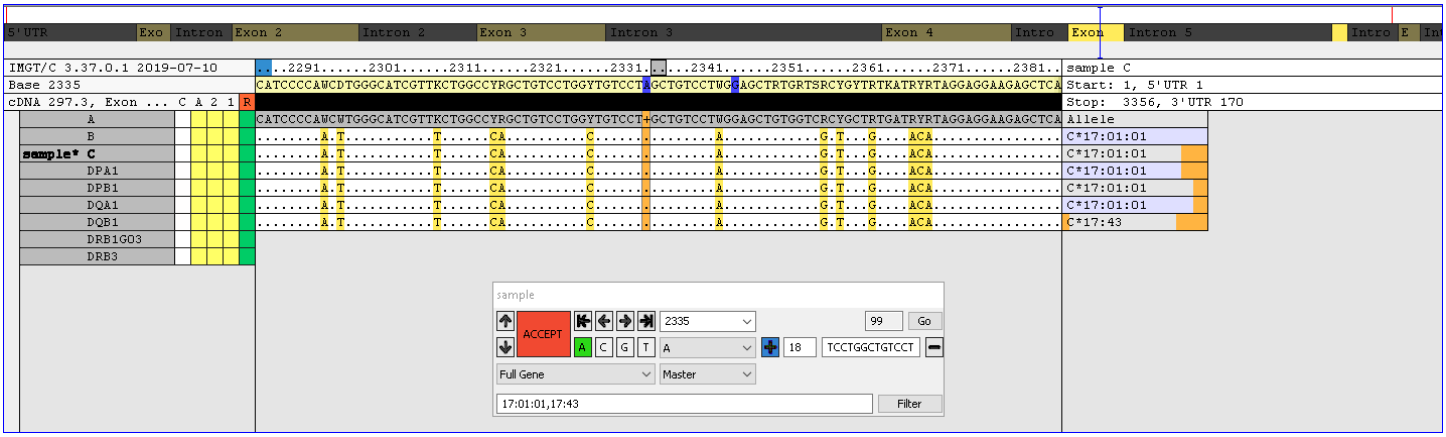


Fig 5. By aligning the reference sequences for C*17:01:01 and C*17:43 we can see where TruSight HLA Assign has positioned the inserted sequence.

As Trusight HLA Assign cannot differentiate the sequences inserted, the insertion at position 2335 is not indicated as a mismatch in this alignment. TruSight HLA Assign does not show the inserted sequence for each reference allele. The sequence of the insertion for the sample will be displayed in the navigator.

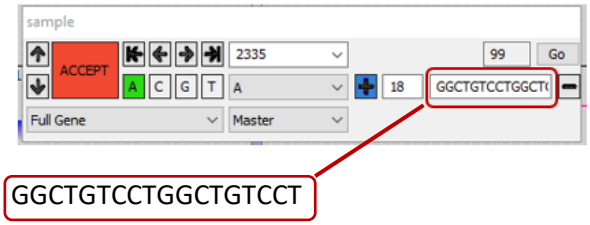


Fig 6. Inserted sequence displayed in the navigator

Align the ambiguous alleles in IMGT and compare with the sequence observed.

| cDNA | 900 | 910 | 920 | 930 | 940 | 950 | 960 | 970 | 980 |
|---|---|---|---|---|---|---|---|---|---|
| C*01:02:01:01 | AGCCG | TCTTCCCAGC | CCACCATCCC | CATCGTGGGC | ATCGTTGCTG | GCCTGGCTG---------- | --------T | CCTGGCTGTC | CTAGCTGTCC | TAGGAGCTGT |
| C*17:01:01:02 | ----- | ---------- | ---------- | --A-T----- | ------T--- | ---CA----TCCTGGCTGT | CCTGGCTG- | ---------- | ---------- | ---------- |
| C*17:01:01:03 | ----- | ---------- | ---------- | --A-T----- | ------T--- | ---CA----TCCTGGCTGT | CCTGGCTG- | ---------- | ---------- | ---------- |
| C*17:01:01:04 | ----- | ---------- | ---------- | --A-T----- | ------T--- | ---CA----TCCTGGCTGT | CCTGGCTG- | ---------- | ---------- | ---------- |
| C*17:01:01:05 | ----- | ---------- | ---------- | --A-T----- | ------T--- | ---CA----TCCTGGCTGT | CCTGGCTG- | ---------- | ---------- | ---------- |
| C*17:01:01:06 | ----- | ---------- | ---------- | --A-T----- | ------T--- | ---CA----TCCTGGCTGT | CCTGGCTG- | ---------- | ---------- | ---------- |
| C*17:01:01:07 | ----- | ---------- | ---------- | --A-T----- | ------T--- | ---CA----TCCTGGCTGT | CCTGGCTG- | ---------- | ---------- | ---------- |
| C*17:01:01:08 | ----- | ---------- | ---------- | --A-T----- | ------T--- | ---CA----TCCTGGCTGT | CCTGGCTG- | ---------- | ---------- | ---------- |
| C*17:01:01:09 | ----- | ---------- | ---------- | --A-T----- | ------T--- | ---CA----TCCTGGCTGT | CCTGGCTG- | ---------- | ---------- | ---------- |
| C*17:43 | ----- | ---------- | ---------- | --A-T----- | ------T--- | ---CA----TCCTGGCTGT | CCTGGCTG- | -------A-- | ---------- | ---------- |

Fig 7. Alignment of C*17:01:01 and C*17:43 with IMGT v3.38.0.

For further assistance contact CareDx tech support email: techsupport-global@caredx.com