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Evaluation of strategies for DRB1 Sequence-Based Typing

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Department of Pathology, Duke University Medical Center, Durham, NC, USA To establish Sequence-Based Typing (SBT) technology for our clinical DRB1 high resolution typing, we evaluated two different SBT strategies:

Generic amplification SBT and

Group-specific SBT.

20 external blind testing samples from a cord blood project were sequenced using the generic amplification SBT where was one generic multiplex amplification followed by three sequencing reactions including a codon 86 GTG-specific reaction for each sample.

Allele level typing results were obtained immediately in only 4 (20%) of these 20 samples.

5 (25%) samples with ambiguous typing results caused by the polymorphic motifs located between codon 10-14 where the primers bind at. In 11 (55%) samples the cis/trans combination of the polymorphic motifs within the sequencing region of two haplotypes mainly contributed to the ambiguities.

All of these ambiguities could be resolved by performing **additional testing** (e.g. SSOP, SSP, or group-specific SBT)

The group-specific amplification SBT we evaluated contains for each sample. 16 amplification reactions including a positive and a negative control followed by two sequencing reactions

Study of the specificities of the group-specific intron primers indicated that this strategy enables us to obtain immediately allele level typing results in all 20 samples. This conclusion was confirmed by the allele level typing results obtained from groupspecific amplification SBT of 5 selected samples previously typed by generic amplification SBT with ambiguous results.

In conclusion, for allele level DRB1 typing performing the "one-shot"

group-specific amplification SBT appears to be more efficient than running generic amplification SBT

followed by additional testing.