**NGS FULL-LENGTH HLA TYPING USING HOLOTYPE HLATM:**

**OUR EXPERIENCE IN PREPARING FOR 17th IHIWS**

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Next-generation sequencing (NGS) is a powerful tool increasingly introduced into HLA clinical laboratories.The accurate phasing of raw NGS data with reference sequences is prerequisite for correct calling of HLA alleles. Gaps in sequence coverage may result in allele ambiguity. In this context, the forthcoming 17th IHIWS aims among other tasks at providing full-length sequences of HLA genes using NGS on reference panel of cell lines. Two cell panels were typed in Olomouc laboratory using Omixon Holotype/Illumina MiSeq, reagent/platform combination. HLA-A, -B, -C, -DPA1 and -DQA1 loci were sequenced for their entire length, -DPB1 from intron 1 to 3' UTR, and -DRB1, -DRB3, -DRB4, -DRB5 from intron 1 to intron 4. Library preparation included amplicon fragmentation, indexed adaptor ligation and size selection. Prior to sequencing, selected fragments were cloned on the sequencing slide using bridge PCR. Collected reads were exported in fastq format and analysed using Omixon Twin software. The best matching alleles were selected according to alignment statistics and homology to alleles in the IMGT/HLA database. HLA-A, -B, -C, DPA1 and -DQA1 loci were successfully sequenced in all samples. Lower rate of success was obtained for other loci (-DRB1, -DRB3, DRB4,-DRB5 -DQB1, -DPB1). Some novel exon variants were revealed (>20), majority within HLA-B ( ̴75%) and DRB1 ( ̴25%) loci. Validation of our data will be performed within the NGS component of the 17th IHIWS.

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