COMPARATIVE TOLERANCE OF TWO MASSIVELY PARALLEL SEQUENCING PLATFORMS TO COMMON PCR INHIBITORS FOR MISSING PERSON CASES

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Human remains are often challenging to identify as they may be highly degraded, fragmented, burnt, decomposed, or contain inhibitory substances. Massively parallel sequencing (MPS) has emerged as an alternative technology to current CE-based genotyping methods. The purpose of this study was to compare the inhibitor tolerance of two MPS-based systems (Ion Torrent™ PGM and Illumina MiSeq®) specifically developed for forensic use and human identification (HID) purposes.

DNA (1 ng) was spiked with various concentrations of five inhibitors (humic acid, melanin, hematin, collagen, and calcium). The samples were sequenced in triplicate using the HID-Ion AmpliSeq™ Identity Panel and Ion PGM™ (Thermo Fisher Scientific) in parallel with the ForenSeq™ DNA Signature Prep Kit (Primer Mix A) on the MiSeq® FGx (Illumina).

Overall, each MPS system seemed to be more tolerant to some inhibitors than others. Both kits performed well with samples with very low levels of inhibitors. Both systems were tolerant to calcium; however, the AmpliSeq™ panel also performed well with collagen and melanin, but failed when even low levels of hematin were present. The ForenSeq™ kit performed relatively well with four inhibitors, but almost failed to genotype samples spiked with melanin.