DEVELOPMENT AND VALIDATION OF THE INVESTIGATOR 24PLEX QS AND 24PLEX GO! KITS

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We report on the development and validation of two Multiplex PCR kits for genotyping of the expanded CODIS STR loci set: Investigator 24plex QS Kit and Investigator GO! Kit. The former kit is designed for purified DNA from casework and reference samples, the latter kit is optimized for direct amplification of reference samples, like blood or buccal cells on FTA or swabs. For swabs, a five minute lysis protocol is provided to prepare samples for direct amplification.

To verify the quality of the DNA sample and the performance of the PCR, both kits contain a novel Quality Sensor as an internal performance control. The system consists of two internal PCR controls (Quality Sensor QS1 and QS2) located at the boarders of the purple dye channel at 74 bp and 435 bp, respectively. The 74 bp QS1 shows very stable amplification, even in the presence of extremely high inhibitor concentrations (e.g. 1000 µM hematin). In contrast the 435 bp QS2 is more prone to inhibition and typically drops out before the first STR marker drop out is observed. Relative signal heights of QS1 and QS2 can thus be used to indicate inhibition. In case both sensors are unaffected, the sample however shows a ski-slope effect with poor amplification of high molecular weight markers, degradation of the DNA sample is most likely. This information can be used to choose the most appropriate rework strategy.

Both assays use a new 6-dye technology to shorten the overall amplicon length and minimize overlaps of the 23 markers which might lead to errors in data interpretation. The kits feature a very robust gender typing by offering small amplification fragments both for Amelogenin and DYS319, leading to correct gender typing even for difficult samples (degraded or inhibited DNA) of Amelogenin null mutant individuals. The developmental validation of the Investigator 24plex kits based on the revised guidelines of the Scientific Working Group on DNA Analysis Methods (SWGDAM) and the recommendations of the European Network of Forensic Science Institutes (ENFSI) has recently been completed. In a sensitivity study, full profiles were consistently obtained with 125 pg template DNA. First stochastic allele drop outs were observed at 63 pg template. Inhibitor studies revealed full DNA profiles in the presence of up to 200 ng/µl humic acid, 750 µM hematin, 3 mM calcium, 12 mM indigo carmine, 4000 ng/µl tannic acid and 200 ng/µl collagen. The kits are validated for NDIS approval.