A46 DNA Quantification by Real-Time PCR (qPCR) and Short-Tandem Repeats (STRs) Amplification Results

Silvia Zoppis, MD, and Manuela Rosini, MSc, Dept of Legal Medicine, Viale Regina Elena 336, Rome, 00161, ITALY; Alessio D'Alessio, PhD, Dept of Legal Medicine, Largo Francesco Vito,1, Rome, 00168, ITALY; and Carla Vecchiotti, PhD, Dept of Legal Medicine, Viale Regina Elena 336, 00161, Rome, ITALY*

After attending this presentation, attendees will understand: (1) principles of genetic analyses on forensic samples; (2) the importance of a valid quantification technique; and, (3) the issues related to the analysis of low template DNA samples.

This presentation will impact the forensic science community by highlighting the importance of the DNA quantification step in forensic casework in spite of the increasing sensitivity of last generation commercial kits for STR analysis that allow the detection of allelic peaks from extremely low DNA quantities (even with concentrations far below the limit of detection for the specific quantification kit).

Determining the amount of DNA in a forensic sample is fundamental for PCR-based analyses because if on one hand an excessive amount of template may cause the appearance of additional or out-of-scale peaks, on the other hand, a low quantity can cause the appearance of stochastic phenomena affecting the PCR reaction and the subsequent interpretation of typing results. In the common practice of forensic genetics laboratories, the quantification results provided by qPCR assume the role of “boundary line” between the possibility for a given DNA sample to be subjected or not to the subsequent analytical steps, on the basis of an optimal amount of DNA in the range indicated by the manufacturer of the specific commercial kit.

However, some studies have shown the possibility to obtain STR typing results even with an extremely low DNA concentration or, paradoxically, equal to zero. Regardless of the amount of DNA used for the quantification of the testing sample, certain software is able to use the standard curve to calculate concentration values far below the manufacturer's reported optimal detection limit (0.023ng/μL). Consequently, laboratories have to face the critical decision to interrupt the analyses, giving up the possibility to obtain a genetic profile—although partial—or to try the amplification of the extract with the awareness of the interpretation issues that this implies.

The quantification results obtained by qPCR performed on numerous samples from specimens of forensic interest, subjected to DNA extraction using magnetic beads will be presented. Following the quantification step, the extracts were subjected to DNA amplification and STR typing using last generation commercial kits. Samples that showed quantification values below the limit of detection for the method were included in the analysis in order to check the existence of a correlation between the DNA quantification results by qPCR and the possibility of obtaining a genetic profile useful for identification purposes.

In spite of the increasing sensitivity of last generation commercial kits for STR analysis, as demonstrated by the ability to detect allelic peaks from extremely low DNA quantities (with concentrations far below the limit of detection for the specific quantification kit, even corresponding to 0 or “Undetermined”), the results obtained show a correlation between qPCR quantification values and STR typing results. Thus, the qPCR method is confirmed as being a useful and valid instrument for both qualitative and quantitative evaluation of genetic samples for human identification purposes.

Quantification, Real-Time PCR (qPCR), Short Tandem Repeats

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