THE QIAGEN INVESTIGATOR® QUANTIPLEX HYres AS AN ALTERNATIVE KIT FOR DNA QUANTIFICATION

Chantal Frégeau, Royal Canadian Mounted Police, Forensic Science and Identification Services

A new dual DNA quantification kit from Qiagen called the Investigator® Quantiplex HYres kit was made available to the forensic community in 2012 with potential advantages (enhanced sensitivity and accuracy) over some of the current commercially available DNA quantification kits. The Investigator® Quantiplex HYres kit was therefore evaluated with respect to its sensitivity, precision and accuracy, reproducibility, robustness and ability to predict the generation of male STR profiles. This work was undertaken to find a potential replacement for the current quantification kit used in Biology Services.

The limit of quantification (LOQ) and limit of detection (LOD) for the Quantiplex HYres kit for both total human and male DNA content using a 20 µL reaction volume were found to be 0.0049 ng/µL and 0.0003 ng/µL, respectively. Reliable quantification results were also obtained using a 10 µL reaction volume conditions. Standard curves (N=72) prepared using 13 Quantiplex HYres kits representing three different lot numbers were highly reproducible. The Quantiplex HYres kit was able to successfully and accurately detect male DNA in mixtures with male:female DNA ratios of 1:1 up to 1:100. However, the 1:500 (0.02 ng/µL male DNA:10 ng/µL female DNA) and 1:1000 (0.02 ng/µL male DNA:20 ng/µL female DNA) mixture samples were challenging for this kit. The Quantiplex HYres kit was able to accurately quantify the total human and male DNA content in a large spectrum of mock casework samples (N=110) and actual casework samples (N=27 processed using our automated protocol and N=52 processed using our manual organic protocol). There were no instances where a female sample provided a male quantification value and all known male samples showed a result regardless of biological source. In general, there was a good correlation between the male quantification values obtained and the percentage male contribution calculated using the gender marker amelogenin amplified using the AmpF/STR® Profiler® Plus kit. Samples with no detectable male DNA using the Quantiplex HYres kit did not produce an amelogenin signal (single source female profiles) while samples with male results using the Quantiplex HYres kit provided single source male profiles, mixed profiles or single source female profiles (male profile masked by the presence of large amounts of female DNA. A fingernail clipping from a deceased female for which a zero quantification value was provided for male DNA using the AB Quantifiler Duo kit gave a male DNA value of 0.0281 ng/µL using the Quantiplex HYres kit. The amplification outcome using AmpF/STR® Profiler® Plus for this sample indicated a mixture involving a male and a female individual. The percentage of male based on the amelogenin marker was scored at 43%. The male/human ratio for this sample based on the Quantiplex HYres kit was calculated at 37.8%. A 20% difference was noted between DNA quantification values obtained using the Quantiplex HYres kit compared to the AB Quantifiler Duo kit attributed to the specific configuration of the Quantiplex HYres kit (chemistry, target sequence and multicopy number of targets).

Based on these observations, the Investigator® Quantiplex HYres kit represents a reliable and robust replacement for the DNA quantification kit currently used in Biology Services.