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Validation of Quantifiler® Trio DNA Quantification kit in forensic samples

N. Gouveia[a,b], P. Brito[a,c], A. Serra[a], F. Balsa[a,c], L. Andrade[a,c], M. São Bento[a,c], P. Cunha[a], V. Bogas[a,c], V. Lopes[a,c], M.J. Porto[a,c,*]

[a] Forensics Genetic and Biology Service, Central Delegation, National Institute of Legal Medicine and Forensic Sciences, I.P., Coimbra, Portugal
[b] Faculty of Sciences, University of Lisbon, Lisbon, Portugal
[c] CENCIFOR, Forensic Sciences Centre, Coimbra, Portugal

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ABSTRACT

The new quantification kit known as Quantifiler® Trio is introduced as more robust and sensitive compared to the Quantifiler® Duo, providing additional information in the analysis of forensic samples. Validation studies were designed to evaluate the sensitivity of the Quantifiler® Trio kit and its performance with mixture and degraded samples.

1. Introduction

Forensic samples often have low quantity and/or degraded DNA, may contain PCR inhibitors and, particularly in sexual assault samples, a high quantity of female DNA compared to male DNA. These factors can make it difficult to decide whether to continue with autosomal STR analysis, which STR kit to use and how much DNA to add to the reaction [1,2]. The Quantifiler® Trio DNA Quantification kit helps overcome these obstacles through enhanced targets that have been designed to determine the quantity of DNA in highly compromised forensic samples, enabling to assess the level of DNA degradation through a new degradation index functionality which was not possible to achieve with the Quantifiler® Duo [3].

The aim of this study is to test the sensitivity of the Quantifiler® Trio kit and its performance with mixture and degraded samples.

2. Materials and methods

The standard curve was generated through tenfold serial dilutions with the 5 standard concentrations ranging from 50 ng/μL to 0.005 ng/μL.

The sensitivity of the Quantifiler® Trio was tested using serial dilutions of 6 DNA controls to obtain concentrations up to a minimum value of 0.005 ng/μL, which represent the smallest point of the standard curve.

In order to test the detection limit of minor contributor, mixture samples containing 1 ng/μL of male DNA and 1 ng/μL of female DNA were prepared. The ratio of male to female DNA was 1:1, 1:2, 1:5, 1:10, 1:15, 1:20, 1:40 and 1:50.

Casework samples were studied to evaluate the impact of different levels of degradation on genetic profiles determined.

All of the samples were quantified according to the manufacturer's instructions on the 7500 Real-Time PCR System and analyzed using the HID Real-Time PCR Analysis Software v1.2. GlobalFiler® PCR Amplification kit was used in amplified samples, normalized to a standard concentration of around 1 ng/μL.

3. Results

DNA quantities obtained with the Quantifiler® Trio were very similar to the expected quantities for both controls and dilutions (Table 1).

Results for the mixture samples are summarized in Table 2. The measured male to female DNA ratio was very close to the expected values for all ratios tested. Amplification results showed that it was possible to obtain a complete female profile in all ratios, whereas a male profile was not visible from a ratio greater than 1:20.

* Corresponding author. Fax: +351239826132.
E-mail address: mariajoao.porto@dcinml.mj.pt (M.J. Porto).

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Table 1
Quantification results of controls and respective dilutions obtained with Quantifiler® Trio.

Samples	Expected quantity (ng/μL)	Measured quantity (ng/μL)	Samples	Expected quantity (ng/μL)	Measured quantity (ng/μL)
Control 1	10	7.6326	**Control 2**	10	8.8824
Control 1 Dil-1	0.2	0.1372	Control 2 Dil-1	0.2	0.1122
Control 1 Dil-2	0.02	0.0131	Control 2 Dil-2	0.02	0.0133
Control 1 Dil-3	0.01	0.0064	Control 2 Dil-3	0.01	0.0092
Control 1 Dil-4	0.005	0.0038	Control 2 Dil-4	0.005	0.0028
Control 3	0.1	0.0863	**Control 4**	0.1	0.0728
Control 3 Dil-1	0.05	0.0433	Control 4 Dil-1	0.05	0.0261
Control 3 Dil-2	0.025	0.0178	Control 4 Dil-2	0.025	0.0128
Control 3 Dil-3	0.0125	0.0071	Control 4 Dil-3	0.0125	0.0071
Control 3 Dil-4	0.00625	0.0026	Control 4 Dil-4	0.00625	0.0063
Control 5	2	1.6023	**Control 6**	2	2.8847
Control 5 Dil-1	0.2	0.1504	Control 6 Dil-1	0.2	0.1477
Control 5 Dil-2	0.02	0.0136	Control 6 Dil-2	0.02	0.0127
Control 5 Dil-3	0.01	0.0080	Control 6 Dil-3	0.01	0.0052
Control 5 Dil-4	0.005	0.0036	Control 6 Dil-4	0.005	0.0029

Table 2
Quantification and amplification results of mixture samples obtained with Quantifiler® Trio and GlobalFiler® kits, respectively. The concentration of female DNA can be approximated by subtracting the male DNA concentration from the human DNA concentration.

Expected M:F ratio	Quantifiler® Trio Kit				GlobalFiler® Kit	
	Human DNA (ng/μL)	Male DNA (ng/μL)	Female DNA (ng/μL)	Measured M:F ratio	Female profile	Male profile
1:1	0.9887	0.5247	0.4640	1:0.88	Complete	Complete
1:2	1.0563	0.3690	0.6873	1:1.86	Complete	Complete
1:5	1.0492	0.1762	0.8730	1:4.96	Complete	Complete
1:10	1.0941	0.0955	0.9986	1:10.46	Complete	23/24
1:15	1.0835	0.0577	1.0258	1:17.79	Complete	19/24
1:20	1.0380	0.0465	0.9915	1:21.33	Complete	14/24
1:40	0.6743	0.0191	0.6552	1:34.22	Complete	–
1:50	0.8881	0.0157	0.8724	1:55.43	Complete	–

Table 3
Quantification and amplification results of casework samples obtained with Quantifiler® Trio and GlobalFiler® kits, respectively. (a – Degradation index was not calculated due to a significant degradation of the samples.)

Samples	Quantifiler® Trio Kit		GlobalFiler® Kit
	Human DNA (ng/μL)	Degradation Index	Profile
A	0.0356	2.3012	Complete
B	0.0346	14.5539	15/24
C	0.0128	1.0668	Complete
D	0.0172	14.6870	15/24
E	0.0240	2.1259	Complete
F	0.0223	11.5330	8/24
G	0.0405	7.4144	13/24
H	0.0873	6.9828	21/24
I	0.2094	a	12/24
J	0.0290	a	6/24

Table 3 contains results for the casework samples. Samples A and B, C and D, E and F have similar concentrations but different degradation index, while samples G and H have different concentrations but similar level of degradation. For a similar concentration, a higher degradation index resulted in a lower quality of genetic profile. On the other hand, more concentrated samples allowed a more complete profile where the level of degradation was similar. Finally, samples I and J are an example of the impact of extreme degradation in obtaining genetic profiles.

4. Discussion and conclusion

Sensitivity studies showed that the DNA quantities obtained with Quantifiler® Trio were very similar to the expected quantities for each control and dilution. The measured male to female ratios were in agreement with the expected ratios revealing the ability of the Quantifiler® Trio to detect small quantities of male DNA in the presence of high amounts of female DNA. Furthermore, it was possible to conclude that a male profile was not visible from a ratio greater than 1:20 and so Y-STR analysis is recommended in these samples. Also, the results demonstrated that the degradation index information can be used to adjust the quantity of DNA in PCR and predict the quality of the expected profiles.

All of these results showed that Quantifiler® Trio is more informative and sensitive in comparison with Quantifiler® Duo.

Conflict of interest

None.

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