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Validation of Quantifiler™ Human Quantification Kit for forensic casework

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Abstract. A study was carried out to test the suitability of Applied Biosystems Quantifiler™ Human Quantification Kit and validate it for forensic casework. The Quantifiler™ assay was performed using an Applied Biosystems 7900HT Real-time PCR system. The validation exercise comprised five parts: (1) Reproducibility, (2) Sensitivity, (3) Effect of bacterial DNA, (4) Effect of reducing reaction volume, and (5) Back-to-back comparison with Picogreen® quantification assay. DNA extracts generated using a variety of extraction methods from different forensic sample types were used for the validation exercise. After quantification ,the DNA extracts were analysed using SGMplus amplification kits. The PCR products were run on 3100 electrophoresis platforms and the resultant DNA profiles analysed using GeneMapperID analysis software. Quantifiler™ gave reproducible results for samples in the DNA concentration range of 0.1 ng/μL–5 ng/μL. The sensitivity of the assay was demonstrated with DNA concentrations of down to 0.03 ng/μL being detected. The presence of increasing ratios of bacterial DNA had no effect on the specificity of the assay. There was no significant difference in calculated DNA concentrations when Quantifiler™ was run at half the recommended reaction volume. The back-to-back study demonstrated that Quantifiler™ generated SGMplus profiles which were on average of better quality than Picogreen® generated profiles. All extracts for which no SGMplus profile could be obtained had Quantifiler™ DNA concentrations of zero. The number of times a sample requiring a second amplification before an acceptable profile was obtained was three times lower for Quantifiler™ samples compared to Picogreen® samples. The validation exercise demonstrated the suitability of the Quantifiler™ assay for forensic casework. © 2006 Published by Elsevier B.V.

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1. Introduction

A validation exercise was carried out to test the suitability of Applied Biosystems Quantifiler™ Human Quantification Kit for forensic casework. The Quantifiler™ assay was performed using Applied Biosystems 7900HT Real-time PCR system. DNA extracts generated from a variety of extraction methods were used for the validation exercise. After quantification, the DNA extracts were amplified using SGMplus. The PCR products were run on a 3100 electrophoresis platform and the resultant DNA profiles analysed using GeneMapperID analysis software.

2. Reproducibility and sensitivity

Nine saliva stain DNA extracts were quantified with Quantifiler™ with eight replicates. The data shows that as the DNA concentration decreases the amount of variation in sample replicate DNA concentration increases. Quantifiler™ gave reproducible results for samples in the DNA concentration range of 0.1 ng/μL–5 ng/μL.

Sample ID	*n*	Average DNA concentration (ng/μL)	Maximum DNA concentration (ng/μL)	Minimum DNA concentration (ng/μL)	% S.D.
Sample 1	8	1.65	1.73	1.49	5.9
Sample 2	8	0.57	0.79	0.49	19.1
Sample 3	8	0.69	0.77	0.59	9.0
Sample 4	8	0.32	0.39	0.18	21.5
Sample 5	8	0.31	0.34	0.26	10.7
Sample 6	8	0.51	0.60	0.43	12.2
Sample 7	8	0.53	0.67	0.48	11.6
Sample 8	8	0.83	0.94	0.74	9.0
Sample 9	8	0.52	0.60	0.41	11.1

A dilution series (2 ng, 1 ng, 500 pg, 250 pg, 100 pg, 50 pg, and 30 pg) generated from a stock Cambio DNA standard was quantified with seven replicates. Positive Quantifiler™ results were recorded for all samples in the dilution series.

3. Effect of bacterial DNA on Quantifiler™ calculated DNA concentrations

A set of mocked-up mixed samples containing both human and bacterial DNA (mixture ratios 1 : 1, 1 : 10, 1 : 100, 1 : 1000 human DNA to bacterial DNA) were quantified with seven replicates plus a control bacteria-only sample. The presence of increasing amounts of spiked bacterial DNA did not have any effect on the Quantifiler™ calculated DNA concentrations, demonstrating the assay's human specificity.

4. Comparison of SGMplus DNA profiles

One hundred forensic casework samples were dual processed from quantification through to profile analysis using both Picogreen® and Quantifiler™ DNA quantification methods. 1 ng of DNA, calculated according to each method, was used in a 50-μL SGMplus amplification. The profiles were scored as to whether they met the loading criteria for submission to the UK National DNA Database. For 14 out of 100 samples, the

profile score was higher for the Quantifiler™ SGMplus DNA profile than the 'paired' Picogreen® SGMplus DNA profile. 5 of the 100 had samples where the profile score was higher for the Picogreen® SGMplus DNA profile than the 'paired' Quantifiler™ SGMplus DNA profile.

5. DNA concentration as a predictor of profile score

An analysis was carried out to investigate the calculated DNA concentration in samples from which no SGMplus profile was obtained.

Sample type	*n*	Picogreen®		Quantifiler™	
		Number with no profile	Number with measured DNA concentration > 0 ng/μL	Number with no profile	Number with measured DNA concentration > 0 ng/μL
Saliva stain	35	4	4	1	0
Hair roots	5	4	2	4	0
Cellular material	28	3	3	2	0
Total	**68**	**11**	**9**	**7**	**0**

The internal PCR control (IPC) data was analysed to determine whether PCR inhibition was the cause of the failures. The sample IPC cycle threshold values did not deviate from the plate mean IPC cycle threshold providing evidence that there was no PCR inhibition. The data suggest that samples with no human DNA measurable using Quantifiler™ will not generate full or partial SGMplus profiles and can therefore be analysed and can be safely aborted.

The first time-pass rate for Quantifiler™ samples was 92% compared to 76% for Picogreen® samples. The lower repeat rate adds further evidence to suggest that Quantifiler™ DNA concentrations are more accurate than Picogreen® concentrations.

6. Conclusion

Quantifiler™ gave reproducible results for samples in the DNA concentration range of 0.1 ng/μL–5 ng/μL. The sensitivity of the assay was demonstrated with DNA concentrations of 0.03 ng/μL being detected. The presence of increasing ratios of bacterial DNA had no effect on the specificity of the assay. The dual processing of samples demonstrated that Quantifiler™ generated SGMplus profiles were of better quality than their counterpart Picogreen® generated SGMplus profiles. The validation exercise has also demonstrated that Quantifiler™ has operational benefits over Picogreen®. The reduced repeat rate and opportunity to stop samples where no human DNA is detected will improve the efficiency of the laboratory by improving the workflow and reducing the downstream processing costs. On the downside, Quantifiler™ is a more costly and time-consuming (2-h processing time compared to 2 min) quantification assay compared to Picogreen®. These facts need to be considered alongside the increased quality benefits when deciding to change from Picogreen® to Quantifiler™.