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COMPARISION OF TWO DNA EXTRACTION METHODS USING THE RT-PCR

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Abstract:

The rapid development of molecular genetic analysis tools has made it possible to analyze most biological material even they are in a small quantity. Many different extraction protocols are being used to isolate DNA. However not all extraction methods perform equally well with heavily inhibited factors and sometimes is very important to choose an extraction procedure that could extract DNA efficiency and had the ability to minimize the amount of inhibitors co-extracted with the sample.

Two different extraction methods were chosen: Chelex and Organic Extraction. To evaluate the efficiency of the procedures, quantity of DNA is compared using TaqMan Probe & Absolute Quantification method in ABI Prism RT-PCR. The samples used were blood samples.

The results showed that with both methods extraction was efficiency for further analysis, but concentration of DNA was greater extracted with Chelex than it with Organic Extraction.

Compare with Organic Extraction, Chelex is more efficient, more rapid, inexpensive, involves fewer steps and thus fewer opportunities for sample to sample contamination, no hazard chemicals are used.

KEY WORDS:
Extraction, DNA, Chelex, Organic Extraction, RT-PCR.

INTRODUCTION

Biological evidence from crime scenes often provides lower quality DNA or may even require analysis from a single cell. The extraction procedure is crucial step in the process of routine forensic human identification. A variety of DNA extraction methods has been used for forensic DNA analysis procedures. Digestion of body fluid stains using SDS and proteinase K, followed by purification of DNA by extraction with phenol/chloroform and ethanol precipitation, is very successful and is routinely used for forensic samples. This method, however, was found to have limitations when applied to a polymerase chain reaction (PCR)-based DNA typing method used in forensic analysis, specifically when this method was applied to bloodstains, because the presence of hematin in bloodstains, which is an inhibitor of PCR, Buttler MJ, (2011), Nielsen. K, (2008).

Another DNA extraction method was more successful in yielding amplifiable DNA, rather than organic extraction. It was a Chelex 100-based extraction method.

The determination of the quantity of human DNA present in a sample and identification of any possible inhibitors on it prior to amplification with the polymerase chain reaction (PCR) is an important

step in forensic DNA analysis using multiple short tandem repeat (STR) markers. By quantifying all DNA samples before performing DNA profiling PCR, the production of such artefacts can be reduced or completely avoided. The latest development in DNA quantitation is based on the technique of real time PCR. A human real-time PCR absolute quantification method of nuclear DNA based on the TaqMan assay was used to evaluate the efficiency of extracted DNA using Quantifiler® Human DNA Quantification Kit. These quantities are only indicative, because Quantifiler amplifies a small fraction of the hTERT gene, which is located adjacent to the 5p telomere. The main goal of this study is to evaluate various methods of DNA isolation in terms of DNA yield and amplification quality Extraction results depend on how the biological material is handling, transport and storage before to do extraction of DNA.

MATERIAL AND METHODS

The biological material used in this study was human blood. Before analysis the blood has dried in filter paper. The sizes of samples used for extraction were 3x3 mm2. The positive control was fresh blood sample in cotton swab. The negative control was without biological material, just reagents. The substrate control was 3x3 mm2 of filter paper without biological material. Two different extraction methods were examined: Chelex-100 resin (Chelex® 100 sodium form, C7901-100G, Sigma Aldrich) and Organic extraction provided by National Forensic Science Technology Center "President's DNA Initiative, (2006). Quantification and PCR inhibition ratio was measured using Absolute Quantitation method, real-time quantitative PCR assay with a fluorogenic TaqMan® probes targeting the human telomerase reverse transcriptase gene (hTERT) using Quantifiler® Human DNA Quantification Kit (ABI, P/N 4343895). Quantification was performed on ABI PRISM® 7000 Sequence Detection System (AB P/N 4330087) with SDS Software v1.0 Applied Biosystem (2006). This quantification system involves a duplex PCR reaction with two independent sets of PCR primers and TaqMan probes. One primer set and the 6-FAM-labeled TaqMan probe is specific to human DNA, while the other primer set and VIC-labeled Taqman probe targets a synthetic sequence that is spiked into each amplification reaction as an internal positive control (IPC). The 6-FAM signal is monitored by an ABI sequence detection system and quantity can be calculated on the basis of the characteristics of the amplification signal. The amplification plot of the VIC-labeled probe is used to determine if any PCR inhibitors are present in the DNA extracts Hoff-Olsen P et al, (1999).

The real time PCR quantification system Quantifiler was chosen to quantify the DNA extracted from the blood samples because it is accurate over a wide range of DNA concentrations (0.023 ng/μL to >50 ng/μL) and it is also capable of assessing the levels of PCR inhibitory compounds in a DNA extract.

RESULTS AND DISCUSSION

There are analyzed 20 random blood samples and three sets of reference samples for each method (positive, negative and substrate controls). Table 1 shows the quantity and cycle threshold of samples for both methods. Real time PCR quantification results showed that Chelex® 100 extraction technique yielded significantly higher amounts of DNA than the organic-based extraction method. (Table 1). Threshold at analysis settings was at point 0.25 (figure 2, 3, 4). Cycle threshold (Ct) for sample extracted with Chelex with quantifiler human detector was at cycles 28 (table 1 & figure 3), Ct for sample extracted with Organic extraction with quantifiler human detector was at cycles 28 -31 (table 1, figure 3), whereas Ct for IPC detector for both methods was between cycles 27, 28 & 29 (figure 2). There was no any significantly difference for the presence of inhibitors but the Ct threshold of IPC detector for samples extracted with Organic method was higher that Ct of IPC extracted with Chelex.

Table I. Quantity of DNA isolated with Chelex and Organic extraction methods

Chelex extraction method				Organic extraction method			
Samples	Sample size	Cycle threshold	Concentration ng/µl	Samples	Sample size	Cycle threshold	Concentration ng/µl
1	3x3 mm²	28	1.15	1	3x3 mm²	29	0.39
2	3x3 mm²	28	1.06	2	3x3 mm²	29	0.4
3	3x3 mm²	28	1.42	3	3x3 mm²	30	0.38
4	3x3 mm²	28	1.32	4	3x3 mm²	31	0.3
5	3x3 mm²	28	1.29	5	3x3 mm²	29	0.4
6	3x3 mm²	28	1.67	6	3x3 mm²	29	0.99
7	3x3 mm²	28	0.9	7	3x3 mm²	28	0.9
8	3x3 mm²	28	0.97	8	3x3 mm²	28	0.88
9	3x3 mm²	28	0.69	9	3x3 mm²	28	0.78
10	3x3 mm²	28	1.24	10	3x3 mm²	28	0.51

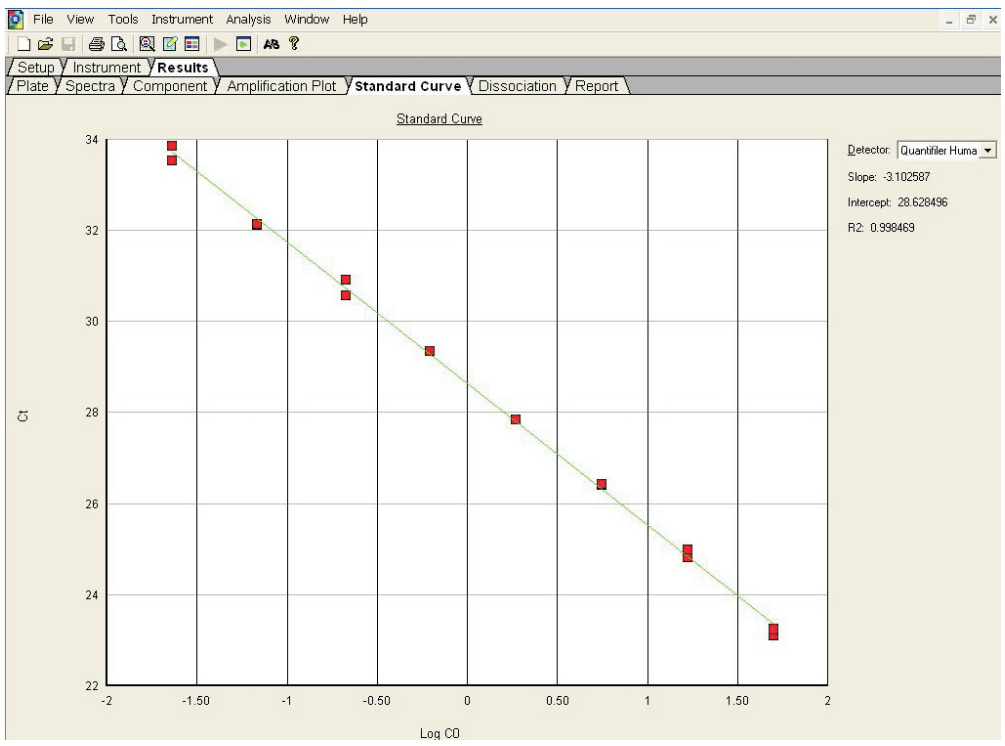

Figure 1. Standard curve of the run performed

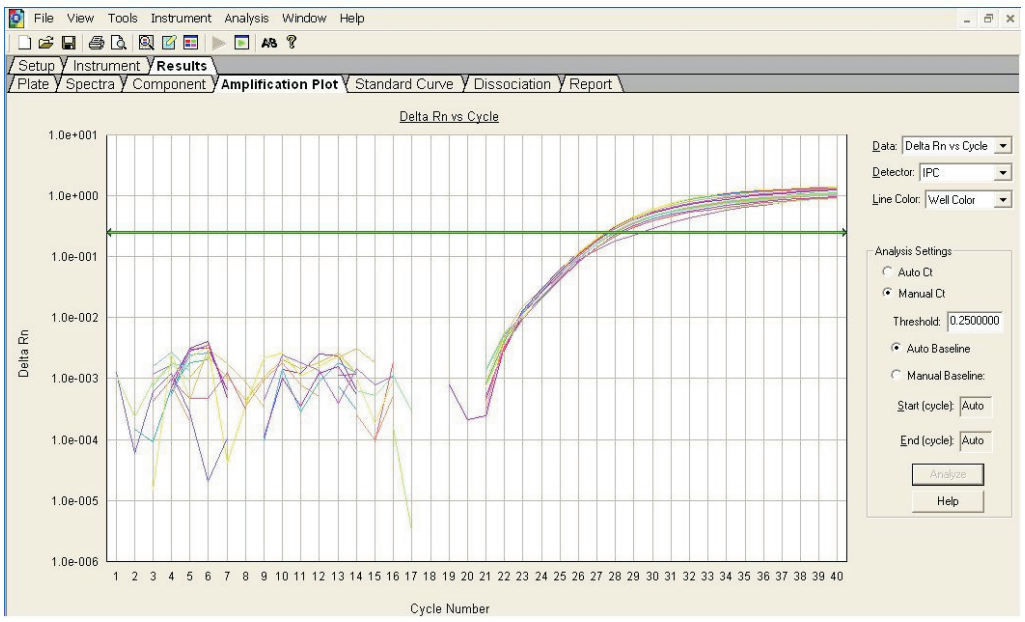

Figure 2. Amplification plot of samples with IPC detector (VIC)

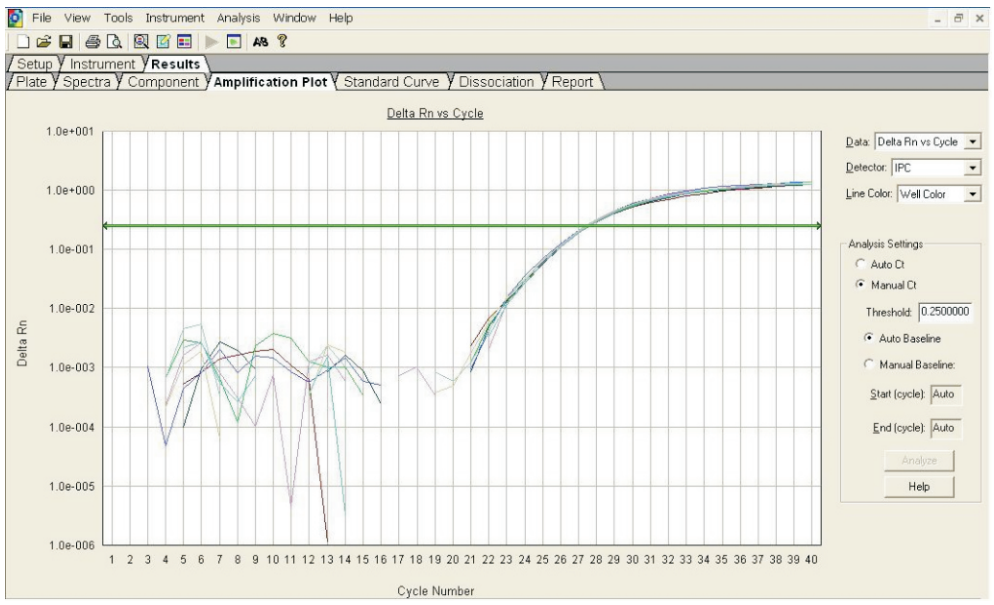

Figure 3. Amplification plot of samples with quantifiler human detector (6-FAM) extracted with Chelex

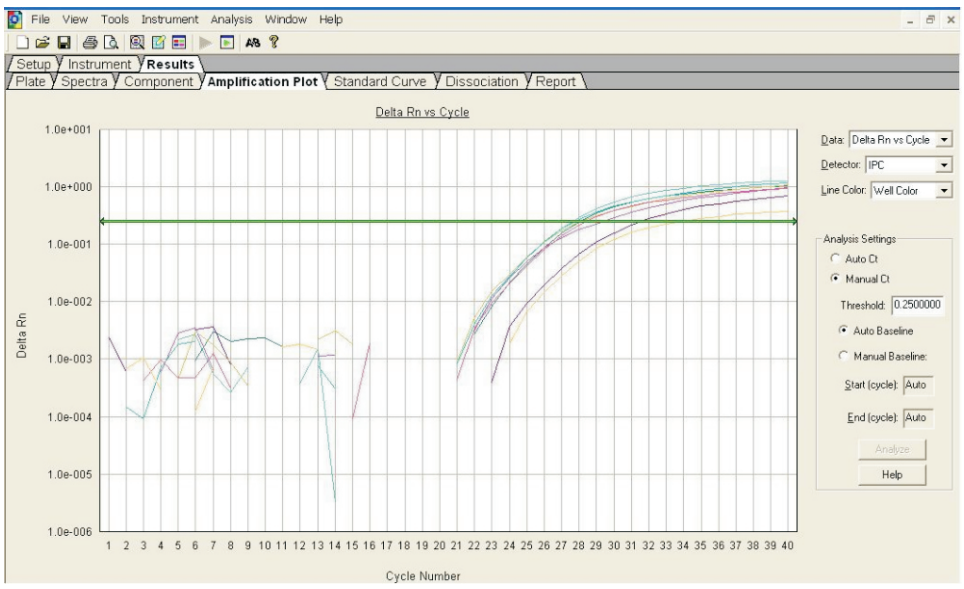

Figure 4. Amplification plot of samples with quantifiler human detector (6-FAM) extracted with Organic extraction

The financial cost of each method was roughly calculated by the summation of the current local prices of both the different chemical solutions specific to each method and of the commercial kits utilized. Stock chemical solutions, disposable parts or the electricity consumption were not included in the calculations. Chelex-100 resin showed to be better in all aspects. Buttler MJ, (2011), Davoren et al, (2007) and Thompson J, (2009) conducted a similar study and arrived the same results.

CONCLUSIONS

As a conclusion, the Chelex-100 resin extraction method had the highest amount of DNA recovered and no inhibitors found in the extracted samples and demonstrated the best amplification results. Chelex-100 resin extraction method examined in this study was proven to be more effective in providing higher quantity of DNA, no level of inhibitors, more rapid and involves fewer steps and thus fewer opportunities for sample to sample contamination, no hazard chemicals use. The silica based method is less expensive than the phenol–chloroform method as well. The organic extraction method is time consuming, involves more steps which increase the possibility for cross contamination of samples, it use the hazard chemicals which can cause the mutagen and carcinogen effects for the analysts who works with it. Those facts make the silica procedure the method of choice. Same conclusions arrives Buttler MJ, (2011), Dixon et al, (2006), Dorak T, (2006), Thompson J, (2009).

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