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RESEARCH

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Carrier RNA is a key factor affecting fully integrated short tandem repeats profiling in challenging forensic samples models

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Abstract

Background: Short tandem repeats (STRs) are used today to provide discriminatory power for DNA fingerprinting. The present results showed that different factors may affect STR profiles in challenging samples including DNA quantity, DNA quality, PCR inhibitors and storage time. In the present study, blood stain samples were applied on two types of fabrics (black cotton and denim) to compare the efficiency of two different DNA-extraction methods (automated magnetic based beads method (EZ1), and manual organic method), with and without adding carrier RNA molecules, and to assess the quality and quantity of the extracted DNA and their capabilities for producing reportable STR-profiles in the presence of PCR inhibitors at two different storage times.

Results: Carrier RNA caused a dramatic increase in DNA recovery from black cotton or denim using EZ1 in contrast to organic method. EZ1 was found to be preferred than organic, especially when a time passed over, while organic method was preferred when samples are available in small quantities. In addition, using carrier RNA within the organic method steps showed no improvement in STR profiling. EZ1 with carrier RNA was preferred for bloodstained samples on fabrics with textile dyes (black dye or denim indigo), especially when stored for a long time.

Conclusions: Denim was found to be more problematic than black cotton due to presence of challenging inhibitors (indigo dye). DNA concentration, storage time and types of fabrics are key factors for choosing the appropriate extraction method for reportable STR profile. Using EZ1 with carrier RNA gives less dropout profile than not using it, or when using organic method even in presence or absence of carrier RNA. Anyway, innovation of more sensitive, more robust analytical protocols could result in a better understanding of these inhibitory samples.

Keywords: EZ1, Organic method, STR profile, Carrier RNA, Bloodstain

Background

Evidence analysis, DNA extraction, PCR amplification of specific loci, and evaluation of amplified target loci are all parts of the standard genotyping workflow in forensic DNA research. DNA extraction, good DNA quality and efficient amplification of target loci all are coordinated operations in achieving excellent portable genotype profile, which is our study ultimate target.

Since the description of the STR utilities in human identification in 1990s, developing robust amplification has been a top priority in improving the genotyping workflow for challenging difficult samples with a higher number of STR loci to achieve greater discrimination power, improve capillary electrophoresis systems, and professional data analysis (Hara et al., 2015).

Factors affecting the efficacy of short tandem repeat amplification (STR) of poorly preserved samples are commonly identified but scarcely assessed. For example, it is a problematic to efficiently amplify poorly preserved PCR samples, thus successful amplification is dependent on several factors, including the amount of recoverable

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DNA, the degree of DNA damage, and the existence of inhibiting agents. In addition, the chemistry and methods used to extract and amplify DNA may have a direct effect on the efficiency of the amplification process (Frégeau et al., 2006). In order to increase the reliability and efficacy of the study, it is essential to analyze the stochastic effects attributed with the analysis of samples with low DNA concentrations including compromising factors.

Common methods of DNA extraction, as an instance, Chelex and organic phenol–chloroform, have been widely and consistently used in forensic laboratories for a long time, and are still the favored methods for some sample types (Lee & Shewale, 2006), while EZ (advanced and biorobot) has succeded in removing several inhibitors of PCR, which are often alongside with the organic method of DNA extraction (Brevnov et al., 2009; Frégeau et al., 2006, 2008; Haak et al., 2008; Montpetit et al., 2005; Scherer et al., 2009; Witt et al., 2012). Many laboratories still depend on Chelex (Walsh et al., 2013) or organic methods (Sambrook, 1989), especially for the extraction of DNA from forensic trace evidence. The requirement to isolate pure DNA without the co-extraction of polymerase inhibitors or other PCR inhibitory components is standard for all extraction methods (Alaeddini, 2012; Wilson, 1997). These inhibitors include fabric dyes like indigo from denim, heme from blood (Broemeling et al., 2008), humic acids from soil (Lakay et al., 2007; Tsai & Olson, 1992) and melanin from hair samples (Eckhart et al., 2000). Depending on the extraction procedure and sample matrix used, the amount of inhibitor co-extracted can vary (Broemeling et al., 2008).

Based on the fabric and its exposure to blood, soil, etc., forensic evidence samples like fabric may contain a number of PCR inhibitory components in various forms. The effectiveness of the amplification process is thus impaired, leading to an increased incidence of pronounced heterozygotes imbalance, allelic dropout, stutter, and non-specific artifacts. (Vallone et al., 2008).

Furthermore, while a sample can be taken from a subject and stored right away, there will always be a forensic sample lag between the investigation of samples on the scene and their collection and processing. It is important to find out whether the delay would affect the extracted DNA's consistency or quantity, and to figure out the best method to be used in such casese (Halsall et al., 2008).

Several techniques have recently been available to potentially enhance DNA recovery, like using carrier molecules such as carrier RNA to improve DNA extraction from microfluidic-based silica monolith (Parys-Proszek et al., 2008). In commercially available Qiagen DNA kits, the addition of carrier RNA to the extraction matrix raises the amount of DNA recovered during the extraction process by an average of 24%. (Morling, 2009). The yields of DNA obtained through robotic extraction in the presence of the carrier RNA were comparable to those obtained through organic extraction without addition of carrier RNA. Carrier RNA was found to assist in the collection of degraded or low quantities of precipitate DNA (Shaw et al., 2009).

Accordingly, in this research, authors evaluated and compared the capability of an automated method of DNA extraction and a manual method with or without addition of carrier RNA molecule to retrieve the maximum amount of DNA possible for fully integrated STR profile amplification. DNA has been extracted from challenging forensic evidence bloodstain samples applied on two types of fabrics dyes "black cotton and denim," at two interval times (1st day & 14th day).

Methods

Samples

Four unrelated healthy volunteers provided fresh venous blood samples using ethylene diamine tetra acetic acid (EDTA) Vacutainer TM tubes.

For control samples, blood samples were collected then DNA has been extracted at two interval times (1st day and 14th day) to help assess the contributing factor and comparing the reliability of the resulting blood STR profiles.

Fabric and stain preparation

Two sets of four cuttings (eight pieces) of approximately $1cm^2$ were excised manually using sterile scalpels from each fabric sample, they were then irradiated by UV for 20 minutes to destroy any extraneous cellular material before stain deposition. 200µl of blood was applied to each fabric before drying at room temperature. The first set (four pieces) of fabric samples was extracted a day after collection and the second set after 14 days. Where each set (with four cuts) was treated as following; one cut for manual extraction with carrier RNA and the other cut without carrier RNA, the third cut for automated DNA extraction with carrier RNA and the fourth one for automated DNA extraction without carrier RNA.

DNA extraction using organic method

To each sample (whole blood from control samples or bloodstained fabrics) 500µL of STE buffer (Sodium Chloride-Tris-EDTA buffer) for extraction (100 mM NaCl,10 mM Tris-Cl, pH 8.0, 1 mM EDTA) plus 20µl of proteinase K (20mg/ml) were added, vortexed, and incubated at 56 °C overnight with agitation in a Thermomixer. 1µl of carrier RNA (included in the kit) was added to half of the sample's volumes, while the other halves continued to be extracted without adding carrier RNA. An equivalent amount of buffered Phenol (pH 8.0) was added and then

centrifuged at top speed for 10 minutes. The aqueous (upper) phase was transferred to a new 0.5 ml chloroform microcentrifuge tube and centrifuged for 10 minutes at full speed. The aqueous (upper) phase was transferred to a new tube, then 210µl ethanol 100% was added, then vortexed vigorously. Each sample was applied to Nucleospin Tissue column (Machery-Nagel-Germany), and centrifuged for 1 minute at 11,000xg, then the flow-through was discarded. The silica membrane was washed twice with distilled water for 1min. The pure DNA was eluted with 200µl TE buffer.

Automated DNA extraction using Qiagen EZ1 advanced

Whole blood from control samples was used directly for extraction, while prior to extraction, bloodstained fabric samples were pre-treated by placing them in a 2 ml sample tube. (Sarstedt, Numbrecht Germany), followed by the addition of 190 µl G2 buffer (it was possible to increase the G2 buffer according to the substrate), and 10 µl of proteinase K tube (Numbrecht Germany) (Qiagen GmbH, Hilden, Germany). After thorough mixing, all samples were incubated for 15 minutes at 56 °C. Every 5 minutes, the incubation stage was interrupted by a mixing step. Half of the sample volumes were mixed with 1µl of carrier RNA (included in the kit), while the other halves continued to be extracted without adding carrier RNA. The DNA was extracted with EZ1 according to the protocol of manufacturer and then eluted with 200 µl TE.

Quantification and qualification of DNA extract

The Quantity of DNA was measured using Nanodrop spectrophotometer, and the blank was carrier RNA to exclude its concentration from the calculated DNA concentration.

Amplification and genotyping

The investigator ID plex Plus Kit is a multiplex human identification kit that comprises the 13 CODIS core loci (Combined DNA Index System), D2S1338, D19S433, and the gender specific Amelogenin (ID-Plex PCR Amplification Kit). 1µL of each PCR products was combined with 14 µl formamide and size standards (BTO) in a 96-well electrophoresis plate (AB) on a Genetic Analyzer (ABI Prism 3500 Genetic Analyzers) employing ABI software (DATA Collection, Gene Mapper ID-X Analysis, version 3.5). PCR amplification was performed according to the manufacturer's recommendations in Veriti 96-well PCR System (Applied Biosystems), using the investigator ID-Plex plus kit (Qiagen). Positive and negative controls were enclosed during the amplification step. STR Analysis with ID-Plex PCR Amplification Kit (Qiagen) was used to evaluate the quality of the extracted DNA. Only alleles with peak heights lower than 150 Relative Fluorescence Units (RFU) and less than 60% heterozygote balance (PHR) were deemed unreliable.

Statistical analysis

The present statistical analyses were executed by aid of Statistical Package for Social Science (SPSS) software version 22. Data were displayed as mean ± standard error. MANOVA test (multiway analysis of variance) was performed to study the effect of the experimental factors (fabric, time, method, and carrier RNA) on the studied parameters. Duncan's test was applied to show similarities among the different methods and fabrics. Independent t-test was applied to illustrate the statistical difference between the values at the day 1and day 14**.**

Results

DNA concentration

Organic extraction of DNA from the two types of fabrics (cotton (F1) and denim (F2)) under study showed significant elevation ($P > 0.05$) of DNA concentration with or without addition of carrier RNA (F1(28.45, 36.61) & F2 (24.16, 26.82), respectively) than that recovered from EZ1 method (F1 (6.91, 1.81) & F2 (8.08, 3.73), respectively), (Table 1 & Fig. 1). Although, the addition of carrier RNA during EZ1 method increased the DNA yield, using carrier RNA with organic method did not significantly ($P > 0.05$) increase the yield of DNA. Generally, the concentration of DNA extracted at the 1st day was higher than that extracted after 14 days with no significant difference between presence or absence of carrier RNA (Table 1 & Fig. 2).

Average peak height (RFU)

RFU is an indication of a reportable STR profile. The present data showed that the average peak height (RFU) of STR genotyping via EZ1was higher than that via organic method (Table 1 and Fig. 3). For F1, EZ1 method with or without addition of carrier RNA showed a higher significant elevation ($P < 0.05$) of the average peak height (RFU) of all sixteen loci than that of organic method "with or without carrier RNA" at both time intervals (Table 2). That was the case for F2 using carrier RNA except for D2S1338, D19S433, respectively (Table 2), While without carrier RNA, only few loci (Amelogenin, TH01, D13S317, FGA) were found to be with higher average height peak (RFU) when compared with that of the organic method "without carrier RNA" (Table 2, Fig. 4).

The profiles from the EZ1 samples at 1st and 14th day displayed an overall more improved interlocus peak balance (RFU) ($P < 0.05$) than those from the organic ones (Table3, Fig. 5).

Table 1 DNA concentration (ng/μL) and average RFU of alleles A1 & A2 of STR profiles resulted from DNA extracted from 2 types of bloodstain fabrics (F1: balck cotton and F2: denim) using two different extraction methods (EZ and Manual). with and without carrier RNA, at two-time intervals (1st day and 14th day)

Time (d)	Fabric (F)	Methods	Carrier	DNA conc	Average RFU
1	1	EZ	With	6.91 ± 1.10^{ab}	7730.28 ± 793.52^{e}
	1	EZ	Without	1.81 ± 0.28^{a}	5152.50 ± 473.81^{d}
	1	Manual	With	28.45 ± 8.25^{cd}	483.36 ± 29.19^{a}
	1	Manual	Without	36.61 ± 10.31^{d}	792.89 ± 51.72^{a}
	2	EZ	With	8.08 ± 1.81^{ab}	5948.63 ± 1263.08^{d}
	2	EZ	Without	3.73 ± 1.09^{ab}	4194.53 ± 702.01^{cd}
	2	Manual	With	24.16 ± 5.47^{cd}	2254.53 ± 199.95^{ab}
	2	Manu al	Without	26.82 ± 8.10^{cd}	2729.61 ± 709.00^{bc}
14	1	EZ	With	3.64 ± 1.03^{ab}	6001.54 ± 707.93^{d}
	1	EZ	Without	1.29 ± 0.41^{a}	4431.41 ± 779.89^{cd}
	1	Manual	With	14.59 ± 3.83^{bc}	405.71 ± 73.12^{a}
	1	Manual	Without	16.54 ± 3.02^{bc}	465.71 ± 138.38^{a}
	2	EZ	With	3.18 ± 0.87^{ab}	4304.73 ± 783.08^{cd}
	2	EZ	Without	1.81 ± 0.28^{a}	1252.60 ± 251.76^{ab}
	2	Manual	With	17.70 ± 4.34^{bc}	733.91 ± 75.66^{a}
	2	Manual	Without	13.52 ± 4.93^{bc}	767.39 ± 27.14^{a}

In the same column, means marked with the same superscript letters are insignificantly different ($P > 0.05$), whereas those marked with different ones are significantly different ($P < 0.05$)

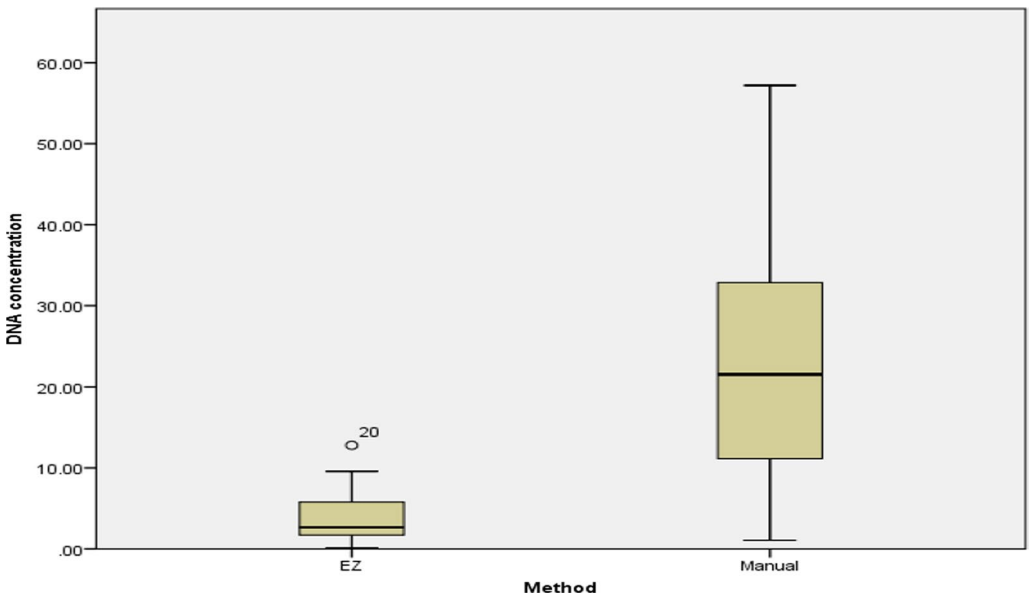

Fig. 1 Box plot comparing DNA concentration of the extracted DNA from bloodstain deposited onto both fabrics using EZ1 and Organic (manual) methods

Peak height ratio (PHR)

STR profiles produced acceptable intralocus heterozygous peak balance (PHR) ranges between (79%-100% and 67%-99%) in (F1 and F2), at 1st or 14th day storage time intervals, respectively. Using EZ1 with carrier RNA, loci with heterozygous imbalance appeared only in F2 in D19S433 (38%), D16S539 (47%) at the 14th day (Table 3). Using EZ1 without carrier RNA resulted in PHR ranges between (82%-99% and 63%-97%) in F1 and F2, at 1st or 14th day, respectively, and loci with heterozygous

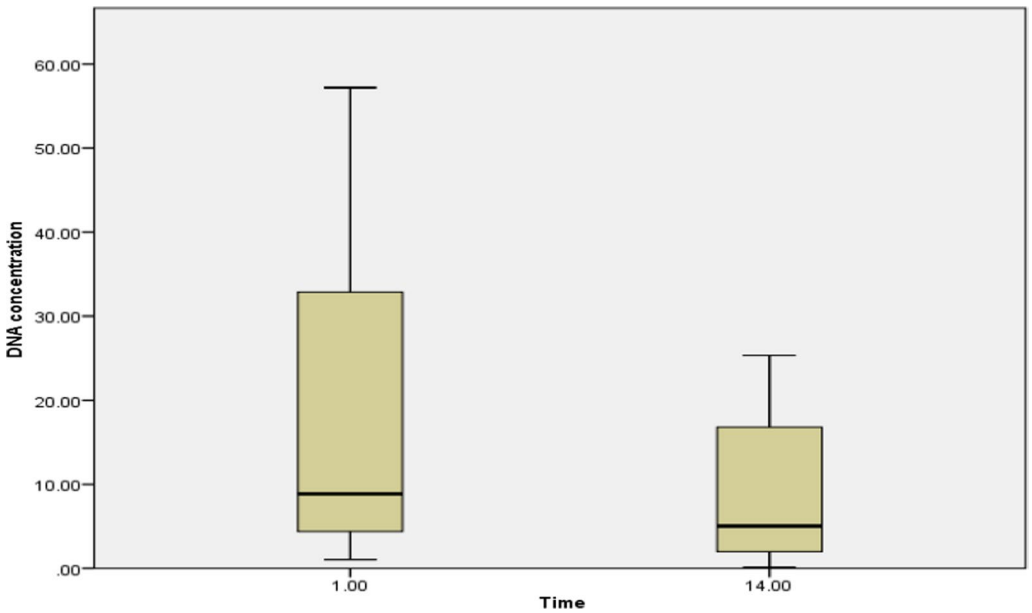

Fig. 2 Boxplot comparing DNA Concentration of the extracted DNA at the 1st day and the 14th day

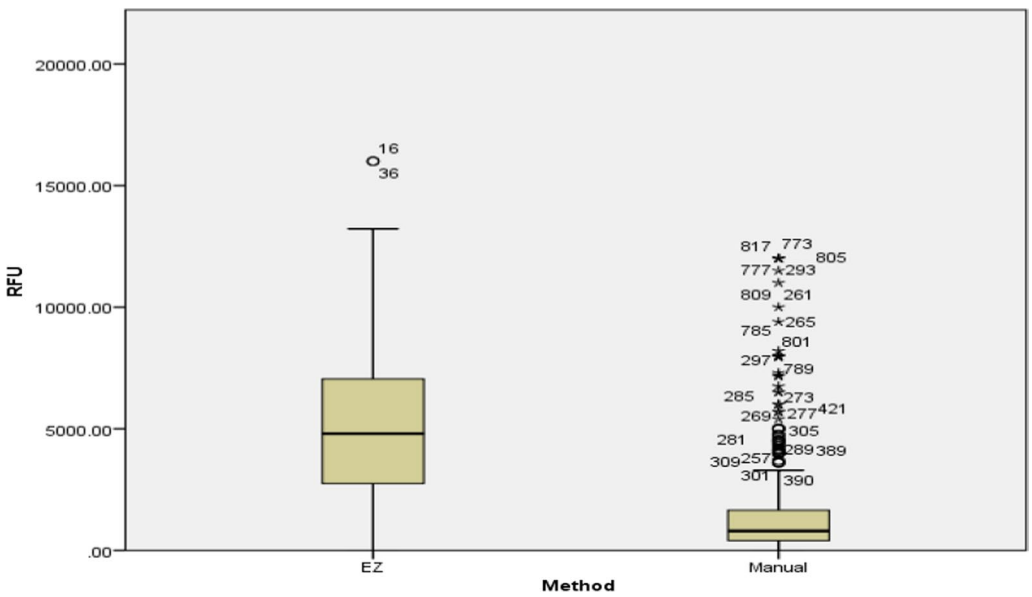

Fig. 3 Boxplot comparing average peak height (RFU) of STR genotyping of the extracted DNA from both EZ1 and manual (Organic) methods

imbalance appeared in D13S317 (32%) at the 14th day only in F2 (Table 3). On the other hand, acceptable intralocus heterozygous peak balance (PHR) has been detected to be within ranges (65%-94%) and (60%-96%) in F1 and F2 loci respectively, using organic method with carrier RNA. In F1, loci with heterozygous imbalance appeared in TH01 (40%), D16S539 (31%), and CSF (53%) at the 14th day, while in F2, they appeared in D19S433 (47%) and D16S539 (47%) at the 1st day (Table 3). Using organic method without carrier RNA, the acceptable intralocus heterozygous peak balance (PHR) showed ranges between (60%-98% and (63%-97%) in F1 and F2, at 1st and 14th day storage time intervals, respectively, and loci with heterozygous imbalance appeared in D3S1358 (58%), D2S1338 (45%), and CSF(50%) at the 1st day, and D3S1358 (53%) at the 14th day in F1, while in F2, they

Table 2 Comparison of average height peak (RFU $*10^3$) for each locus of STR profile of DNA extracted from two bloodstain fabrics, black cotton (F1) and denim (F2) using EZ1 extraction method and organic method at 1st day and 14th day

		Carrier	With carrier				Without carrier			
		Method	Ez		Organic		Ez		Organic	
		Fabric	F1	F2	F1	F2	F1	F2	F1	F2
Amel	Time	1	9.17 ± 1.13^{C}	7.71 ± 1.44^{C}	0.46 ± 0.01^{A}	2.92 ± 0.86^{B}	7.76 ± 1.11^{C}	6.95 ± 0.77^{C}	0.80 ± 0.10^{A}	3.45 ± 0.60^{B}
		14	7.20 ± 1.18^{C}	5.52 ± 0.87^{BC}	0.72 ± 0.25^{A}	$0.61 \pm 0.04^{A*}$	$4.13 \pm 1.07^{B*}$	$1.04 \pm 0.42^{A*}$	0.78 ± 0.26^{A}	$0.41 \pm 0.11^{A*}$
TH01	Time	1	8.20 ± 0.98^{D}	7.95 ± 1.35^{CD}	0.97 ± 0.11^{A}	3.14 ± 0.54^{B}	5.53 ± 0.53^{BC}	7.25 ± 0.38^{CD}	1.33 ± 0.16^{A}	4.73 ± 1.22^{B}
		14	8.00 ± 0.39^{C}	5.90 ± 0.89^{B}	0.71 ± 0.10^{A}	$1.30 \pm 0.27^{A*}$	5.98 ± 1.36^{B}	$2.38 \pm 0.69^{A*}$	$0.58 \pm 0.16^{A*}$	$0.90 \pm 0.17^{A*}$
D3S1358	Time	1	10.43 ± 0.59^{E}	7.18 ± 1.15^{D}	0.48 ± 0.07^{A}	2.91 ± 0.55^{BC}	7.56 ± 1.01^{D}	4.70 ± 0.64^{C}	0.71 ± 0.14^{A}	3.68 ± 1.11^{C}
		14	$8.01 \pm 0.94^{C*}$	5.66 ± 1.48^{B}	0.63 ± 0.19^{A}	$0.93 \pm 0.22^{A*}$	5.62 ± 0.31^{B}	4.95 ± 0.46^{B}	$0.32 \pm 0.03^{A*}$	$1.14 \pm 0.39^{A*}$
VWA	Time	1	11.03 ± 1.76^{D}	8.32 ± 2.29^{CD}	0.58 ± 0.14^{A}	2.80 ± 0.79^{AB}	5.94 ± 1.17^{BC}	5.54 ± 2.33^{BC}	0.97 ± 0.30^{A}	3.18 ± 0.82^{AB}
		14	8.83 ± 1.59^{B}	7.03 ± 2.30^{B}	0.52 ± 0.19^{A}	$0.84 \pm 0.27^{A*}$	6.04 ± 2.19^{B}	$1.49 \pm 0.42^{A*}$	0.33 ± 0.07^{A}	$0.81 \pm 0.27^{A*}$
D21S11	Time	1	9.71 ± 1.24^{D}	7.52 ± 1.29^{CD}	0.49 ± 0.10^{A}	2.28 ± 0.28^{B}	6.52 ± 0.51^{C}	3.90 ± 1.54^{B}	0.71 ± 0.10^{A}	1.89 ± 0.38^{AB}
		14	8.23 ± 0.98^{C}	$4.84 \pm 1.21^{B*}$	0.30 ± 0.07^{A}	$0.83 \pm 0.13^{A*}$	5.33 ± 1.14^{B}	$1.30 \pm 0.13^{A*}$	0.42 ± 0.19^{A}	$0.79 \pm 0.19^{A*}$
TPOX	Time	1	6.40 ± 1.09^{D}	6.25 ± 1.37^{D}	0.54 ± 0.08^{A}	2.51 ± 0.25^{AB}	3.87 ± 0.72^{BC}	4.84 ± 0.53^{CD}	0.76 ± 0.24^{A}	2.99 ± 0.47^{BC}
		14	4.49 ± 1.05^{B}	$3.91 \pm 1.08^{B*}$	0.58 ± 0.15^{A}	$1.17 \pm 0.41^{A*}$	3.59 ± 0.69^{B}	$0.65 \pm 0.14^{A*}$	0.30 ± 0.04^{A}	$0.43 \pm 0.11^{A*}$
D7S820	Time	1	9.49 ± 1.47^{D}	7.16 ± 1.98^{CD}	0.61 ± 0.11^{A}	2.73 ± 0.72^{AB}	7.77 ± 1.53^{CD}	5.43 ± 1.83^{BC}	1.10 ± 0.34^{A}	3.32 ± 0.45^{AB}
		14	8.76 ± 1.38^{B}	5.98 ± 1.96^{B}	$0.26 \pm 0.02^{A*}$	$0.90 \pm 0.38^{A*}$	6.79 ± 1.99^{B}	$1.46 \pm 0.21^{A*}$	$0.34 \pm 0.07^{A*}$	$0.50 \pm 0.02^{A*}$
D19S433	Time	1	5.44 ± 0.84^{C}	5.35 ± 0.95^{C}	0.53 ± 0.11^{A}	2.27 ± 0.31^{AB}	3.66 ± 0.76^{BC}	2.31 ± 0.64^{AB}	1.77 ± 0.92^{AB}	2.43 ± 0.35^{AB}
		14	6.83 ± 0.44^{D}	$2.64 \pm 0.48^{BC*}$	0.50 ± 0.05^{A}	$0.91 \pm 0.17^{AB*}$	4.06 ± 1.48^{C}	1.69 ± 0.55^{AB}	0.42 ± 0.14^{A}	$0.87 \pm 0.25^{AB*}$
D5S818	Time	1	$11.35 \pm .61^{D}$	7.56 ± 1.92^{C}	0.42 ± 0.05^{A}	2.22 ± 0.43^{AB}	8.29 ± 1.14^{CD}	5.00 ± 1.30^{BC}	0.67 ± 0.13^{A}	$1.98 \pm .75^{AB}$
		14	8.88 ± 1.73^{B}	6.28 ± 0.92^{B}	0.26 ± 0.06^{A}	$1.07 \pm 0.17^{A*}$	6.57 ± 2.38^{B}	$1.64 \pm 0.30^{A*}$	$0.14 \pm 0.03^{A*}$	1.08 ± 0.23^{A}
D2S1338	Time	1	3.94 ± 054^{C}	2.63 ± 0.69^{B}	0.66 ± 0.08^{A}	1.50 ± 0.46^{AB}	2.48 ± 0.31^{B}	1.84 ± 0.12^{AB}	0.73 ± 0.16^{A}	$1.70 \pm .42^{AB}$
		14	$2.59 \pm .50^{C*}$	2.27 ± 0.60^{BC}	$0.35 \pm 0.04^{A*}$	0.88 ± 0.18^{A}	2.12 ± 0.73^{BC}	0.89 ± 0.03^{A}	0.68 ± 0.05^{A}	1.33 ± 0.19^{AB}
D16S539	Time	1	7.21 ± 0.65^{D}	5.11 ± 0.82^{C}	0.60 ± 0.09^{A}	1.73 ± 0.28^{AB}	$5.80 \pm .14^{CD}$	2.90 ± 0.79^{B}	0.62 ± 0.12^{A}	$1.51 \pm .29^{AB}$
		14	$4.31 \pm .65^{D*}$	$3.37 \pm .91^{CD*}$	0.43 ± 0.13^{A}	1.13 ± 0.24^{AB}	$3.00 \pm .78^{CD*}$	2.38 ± 0.81^{BC}	0.48 ± 0.13^{A}	$0.85 \pm .28^{AB}$
CSF1PO	Time	1	5.31 ± 0.91^{D}	4.07 ± 0.83^{CD}	0.41 ± 0.08^{A}	1.45 ± 0.37^{AB}	3.53 ± 0.37^{C}	2.74 ± 0.34^{BC}	0.53 ± 0.87^{A}	1.80 ± 0.44^{AB}
		14	$3.55 \pm 0.68^{C*}$	2.63 ± 0.53^{C}	0.31 ± 0.18^{A}	$0.70 \pm 0.80^{AB*}$	3.03 ± 0.80^{C}	1.99 ± 0.82^{BC}	0.40 ± 0.01^{AB}	0.75 ± 0.20^{AB}
D13S317	Time	1	6.78 ± 1.16^{C}	7.26 ± 1.25^{C}	0.41 ± 0.06^{A}	1.68 ± 0.29^{A}	4.34 ± 0.78^{B}	4.13 ± 1.05^{B}	0.74 ± 0.09^{A}	1.20 ± 0.28^{A}
		14	$3.72 \pm 0.64^{B*}$	$2.81 \pm 0.97^{B*}$	0.38 ± 0.17^{A}	0.71 ± 0.09^{A}	3.04 ± 0.94^{B}	$0.66 \pm 0.09^{A*}$	0.45 ± 0.08^{A}	0.82 ± 0.26^{A}
FGA	Time	1	7.94 ± 0.91^{B}	7.98 ± 1.39^{B}	1.20 ± 0.82^{A}	1.83 ± 0.45^{A}	6.30 ± 0.75^{B}	5.38 ± 0.86^{B}	0.97 ± 0.27^{A}	1.96 ± 0.72^{A}
		14	6.99 ± 1.28^{D}	$4.13 \pm 0.94^{BC*}$	0.21 ± 0.06^{A}	0.70 ± 0.11^{A}	5.39 ± 1.26^{CD}	$2.39 \pm 1.06^{AB*}$	$0.32 \pm 0.06^{A*}$	0.63 ± 0.15^{A}
D18S51	Time	1	6.35 ± 1.03^{D}	5.62 ± 1.43^{CD}	0.31 ± 0.04^{A}	1.40 ± 0.32^{AB}	4.89 ± 0.94^{CD}	3.51 ± 0.38^{BC}	0.71 ± 0.83^{A}	1.70 ± 0.44^{AB}
		14	$4.45 \pm 0.83^{C*}$	$3.38 \pm 0.86^{BC*}$	0.30 ± 0.07^{A}	$0.55 \pm 0.13^{A*}$	3.77 ± 0.67^{C}	1.91 ± 0.76^{AB}	$0.29 \pm 0.09^{A*}$	$0.61 \pm 0.10^{A*}$
D8S1179Z	Time	1	4.79 ± 0.51^{DE}	5.28 ± 0.76^{E}	0.20 ± 0.04^{A}	1.43 ± 0.45^{AB}	3.55 ± 0.35^{CD}	3.53 ± 0.44^{CD}	0.63 ± 0.06^{A}	2.31 ± 0.60^{BC}
		14	3.83 ± 0.64^{B}	$2.68 \pm 0.50^{B*}$	0.22 ± 0.06^{A}	$0.38 \pm 0.06^{A*}$	3.25 ± 0.53^{B}	$1.34 \pm 0.58^{A*}$	$0.30 \pm 0.04^{A*}$	$0.44 \pm 0.09^{A*}$

In the same row, means marked with different superscript letters are significantly different ($P < 0.05$), whereas those marked with the same ones are insignificantly different ($P > 0.05$). *: significant difference ($P < 0.05$), as compared to the value at the first day

appeared in D3S1358 (31%), at the 1st day and D3S1358 (39%), and FGA (57%) at the 14th day (Table 3).

Partial STR profiles and dropout alleles

Partial profiles appeared in STR profiles obtained from organic method were more in number than those obtained from EZ1 method particularly in the 14th day (Fig. 7). Dropout alleles include peaks with peak height ratio (PHRs) less than 60% in heterozygous loci, and the peaks that were not called by the genotyping software because they had relative fluorescence unit (RFU) values lower than the AT, which was set by default parameters. The present data showed that dropout appeared mainly in STR profile obtained from EZ1 method" without carrier RNA" in 14th day (Fig. 6). In F1 at 1st day, Using EZ1 with carrier RNA addition, no dropout appeared, while at the 14th day, only locus D8S1179 (49%) showed dropout allele. On the other hand, in F2 at 1st day there was no dropout, while at 14th day, only locus D8S1179 (49%) was considered as a dropout allele (Table 4, Figs. 6, 7).

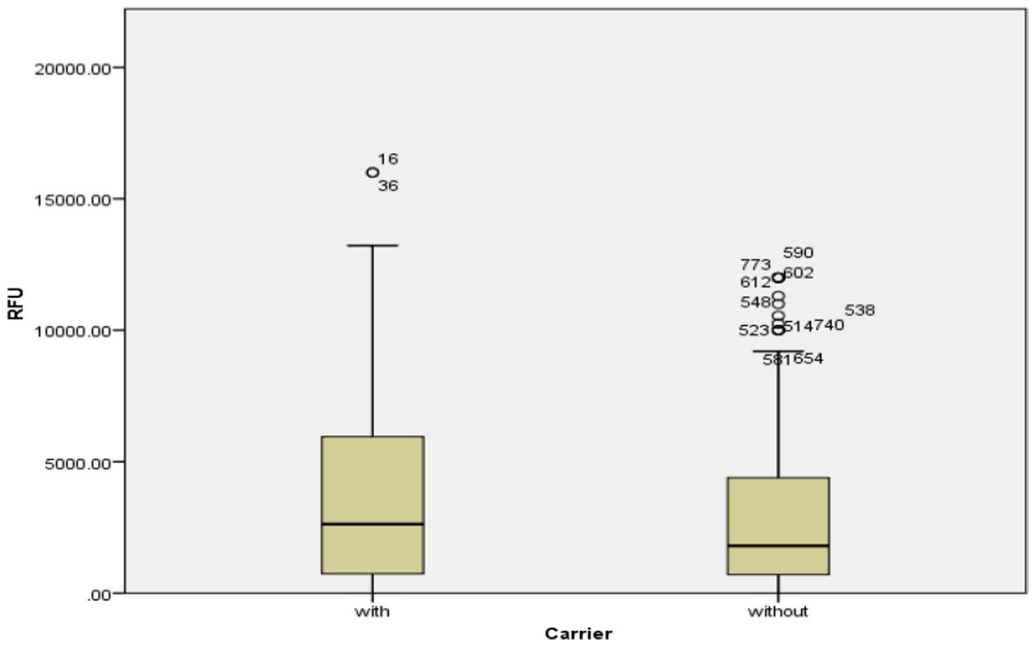

Fig. 4 Boxplot comparing average peak height (RFU) of STR genotyping of the extracted DNA from EZ1 extraction method "with carrier RNA and without carrier RNA"

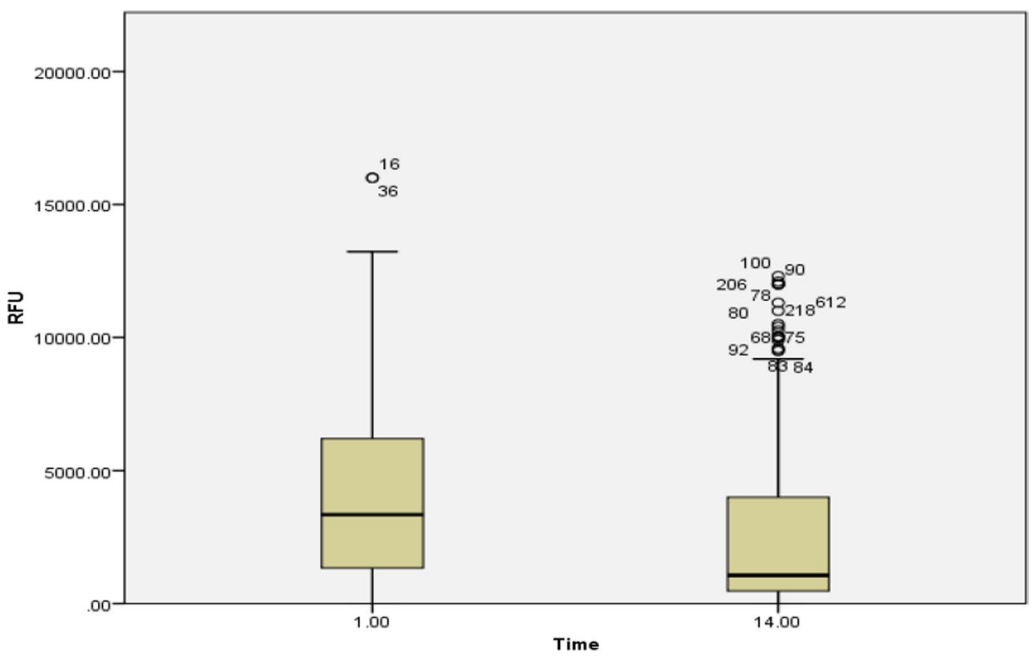

Fig. 5 Boxplot comparing average peak height (RFU) of STR genotyping of the extracted DNA at 1st day and 14th day

In case of absence of carrier RNA using EZ1 in F1, the loci D19S433 (54%) and D8S1179 (49%) at day 1 and 14 were considered dropout alleles, respectively. While in F2 at 1st day, loci FGA(42%), D21S11 (57%), and TPOX (58%), and at 14th day, loci CSF1PO(40%), D8S1179 (50%), D21S11 (0%), TH01 (51%), D21S11 (50%), and TPOX(33%) were considered dropout alleles (Table 4, Figs. 6,7).

Table 3 heterozygous peak ratio (PHR) for each locus from profiles using two extraction methods (EZ1 and organic), "with and without carrier" from two types of fabrics (Black cotton (F1) and denim (F2)), at two storage times (1st and 14th)

		Carrier	With carrier				Without carrier			
		Method	Ez		Manual		Ez		Manual	
		Fabric	F1	F2	F1	F2	F1	F2	F1	F2
Ameglonin	Time	1	0.95 ± 0.03b	0.91 ± 0.04ab	0.69 ± 0.13a	0.80 ± 0.14ab	0.90 ± 0.05ab	0.89 ± 0.06ab	0.93 ± 0.02ab	0.94 ± 0.02b
		14	0.91 ± 0.03ab	0.80 ± 0.14ab	0.90 ± 0.07ab	0.91 ± 0.06ab	0.93 ± 0.04ab	0.94 ± 0.02b	0.94 ± 0.02b	0.70 ± 0.09a*
TH01	Time	1	1.00 ± 0.005b	0.97 ± 0.01b	0.65 ± 0.11a	0.97 ± 0.02b	0.92 ± 0.06b	0.90 ± 0.07ab	0.98 ± 0.001b	0.80 ± 0.03ab
		14	0.83 ± 0.08b	0.97 ± 0.03b	0.40 ± 0.03a	0.61 ± 0.14ab	0.86 ± 0.12b	0.80 ± 0.05b	0.74 ± 0.21ab	0.63 ± 0.08ab
D3S1358	Time	1	0.94 ± 0.001b	0.90 ± 0.01ab	0.90 ± 0.04ab	0.93 ± 0.02b	0.94 ± 0.02b	0.93 ± 0.04b	0.58 ± 0.12a	0.79 ± 0.13ab
		14	0.85 ± 0.01a*	0.93 ± 0.02a	0.76 ± 0.17a	0.75 ± 0.04a	0.93 ± 0.001a	0.79 ± 0.13a	0.53 ± 0.02a	0.64 ± 0.05a
VWA	Time	1	0.90 ± 0.09a	0.96 ± 0.01a	0.83 ± 0.08a	0.67 ± 0.18a	0.96 ± 0.03a	0.91 ± 0.07a	0.90 ± 0.06a	0.90 ± 0.06a
		14	0.86 ± 0.03a	0.67 ± 0.18a	0.83 ± 0.08a	0.81 ± 0.08a	0.98 ± 0.01a	0.90 ± 0.06a	0.92 ± 0.04a	0.84 ± 0.07a
D21S11	Time	1	0.88 ± 0.04a	0.88 ± 0.10a	0.79 ± 0.07a	0.84 ± 0.06a	0.88 ± 0.03a	0.90 ± 0.08a	0.90 ± 0.05a	0.91 ± 0.09a
		14	0.79 ± 0.08ab	0.84 ± 0.06ab	0.79 ± 0.01ab	0.69 ± 0.07a	0.97 ± 0.01b*	0.91 ± 0.09ab	0.90 ± 0.03ab	0.69 ± 0.12a
TPOX	Time	1	0.93 ± 0.05a	0.85 ± 0.09a	0.92 ± 0.05a	0.96 ± 0.02a	0.93 ± 0.04a	0.97 ± 0.01a	0.88 ± 0.07a	0.87 ± 0.06a
		14	0.97 ± 0.01b	0.97 ± 0.03b	0.78 ± 0.14a	0.87 ± 0.07ab	0.98 ± 0.01b	0.86 ± 0.06ab	0.89 ± 0.04ab	0.85 ± 0.07ab
D7S820	Time	1	0.85 ± 0.05ab	0.90 ± 0.04b	0.69 ± 0.05a	0.90 ± 0.07b	0.94 ± 0.03b	0.96 ± 0.02b	0.88 ± 0.06b	0.97 ± 0.03b
		14	0.96 ± 0.01b	0.90 ± 0.07b	0.60 ± 0.14a	0.92 ± 0.06b	0.83 ± 0.08ab	0.97 ± 0.03b	0.94 ± 0.03b	0.86 ± 0.02b
D19S433	Time	1	0.88 ± 0.06b	0.92 ± 0.03b	0.91 ± 0.03b	0.39 ± 0.02a	0.82 ± 0.12b	0.91 ± 0.02b	0.87 ± 0.05b	0.95 ± 0.03b
		14	0.81 ± 0.08bc	0.38 ± 0.02a*	0.90 ± 0.07bc	0.66 ± 0.10b	0.85 ± 0.13bc	0.95 ± 0.03bc	0.96 ± 0.02c	0.90 ± 0.03bc
D5S818	Time	1	0.97 ± 0.02^{a}	0.88 ± 0.05^{a}	0.74 ± 0.17^{a}	0.84 ± 0.09^{a}	0.94 ± 0.03^{a}	0.96 ± 0.02^{a}	0.75 ± 0.09^{a}	0.90 ± 0.07^{a}
		14	0.94 ± 0.01^{a}	0.84 ± 0.09^{a}	0.80 ± 0.07^{a}	0.77 ± 0.10^{a}	0.92 ± 0.03^{a}	0.90 ± 0.07^{a}	0.76 ± 0.10^{a}	0.78 ± 0.09^{a}
D2S1338	Time	1	0.99 ± 0.01^{d}	0.98 ± 0.01^{d}	0.81 ± 0.01^{bc}	0.79 ± 0.06^{b}	0.96 ± 0.02^{d}	0.97 ± 0.02^{d}	0.45 ± 0.04^{a}	0.91 ± 0.04^{cd}
		14	0.98 ± 0.01^{b}	0.79 ± 0.06^{a}	0.90 ± 0.07^{b}	0.96 ± 0.02^{b}	0.99 ± 0.01^{b}	0.91 ± 0.04^{b}	$0.96 \pm 0.02^{b*}$	0.76 ± 0.12^{a}
D16S539	Time	1	0.99 ± 0.01^{b}	0.91 ± 0.03^{b}	0.46 ± 0.03^{a}	0.47 ± 0.09^{a}	0.85 ± 0.08^{b}	0.94 ± 0.01^{b}	0.60 ± 0.08^{a}	0.94 ± 0.03^{b}
		14	0.94 ± 0.05^{d}	$0.47 \pm 0.09^{ab*}$	$0.31 \pm 0.01^{a*}$	0.60 ± 0.03^{bc}	0.91 ± 0.04^{d}	0.95 ± 0.03^{d}	0.76 ± 0.03^{c}	0.83 ± 0.08^{d}
CSF1PO	Time	1	0.97 ± 0.02^{a}	0.97 ± 0.01^{a}	0.92 ± 0.04^{a}	0.94 ± 0.04^{a}	0.99 ± 0.01^{a}	0.92 ± 0.03^{a}	0.96 ± 0.02^{a}	0.97 ± 0.02^{a}
		14	0.95 ± 0.05^{c}	0.77 ± 0.18^{bc}	$0.53 \pm 0.06^{ab*}$	0.81 ± 0.08^{bc}	0.91 ± 0.07^{c}	0.97 ± 0.02^{c}	$0.50 \pm 0.04^{a*}$	0.86 ± 0.09^{c}
D13S317	Time	1	0.96 ± 0.04^{b}	0.99 ± 0.01^{b}	0.83 ± 0.08^{b}	0.90 ± 0.09^{b}	0.93 ± 0.04^{b}	0.94 ± 0.01^{b}	0.83 ± 0.10^{b}	0.31 ± 0.06^{a}
		14	0.92 ± 0.04^{c}	0.90 ± 0.08^{c}	0.74 ± 0.08^{bc}	$0.60 \pm 0.02^{b*}$	0.89 ± 0.05^{c}	$0.32 \pm 0.06^{a*}$	0.84 ± 0.02^{c}	0.39 ± 0.02^{a}
FGA	Time	1	0.93 ± 0.03^{a}	0.96 ± 0.02^{a}	0.80 ± 0.004^{a}	0.80 ± 0.09^{a}	0.89 ± 0.04^{a}	0.77 ± 0.15^{a}	0.76 ± 0.08^{a}	0.92 ± 0.04^{a}
		14	0.97 ± 0.01^{b}	0.80 ± 0.09^{b}	0.88 ± 0.09^{b}	0.94 ± 0.05^{b}	0.92 ± 0.04^{b}	0.92 ± 0.04^{b}	0.86 ± 0.04^{b}	$0.57 \pm 0.08^{a*}$
D18S51	Time	1	0.92 ± 0.03^{b}	0.95 ± 0.03^{b}	0.95 ± 0.04^{b}	0.88 ± 0.05^{b}	0.87 ± 0.05^{b}	0.87 ± 0.02^{b}	0.61 ± 0.17^{a}	0.77 ± 0.11^{ab}
		14	0.96 ± 0.02^{a}	0.88 ± 0.05^{a}	0.94 ± 0.06^{a}	0.66 ± 0.08^{a}	0.91 ± 0.04^{a}	0.77 ± 0.11^{a}	0.70 ± 0.30^{a}	0.73 ± 0.10^{a}
D8S1179	Time	1	0.98 ± 0.01^{b}	0.93 ± 0.04^{b}	0.73 ± 0.13^{a}	0.91 ± 0.04^{ab}	0.93 ± 0.04^{b}	0.90 ± 0.05^{ab}	0.82 ± 0.10^{ab}	0.98 ± 0.01^{b}
		14	0.96 ± 0.01^{b}	0.91 ± 0.04^{b}	0.94 ± 0.05^{b}	$0.66 \pm 0.08^{a*}$	0.97 ± 0.01^{b}	0.98 ± 0.01^{b}	0.98 ± 0.02^{b}	0.91 ± 0.07^{b}

In the same row, means marked with different superscript letters are significantly different ($P < 0.05$), whereas those marked with the same ones are insignificantly different ($P > 0.05$). *: significant difference ($P < 0.05$), as compared to the value at the first day

On the other hand, using organic method with carrier RNA revealed less dropout alleles than not using the carrier. Overall results demonstrated that using EZ1 with carrier RNA gives less dropout than not using it, or using organic method even in presence or absence of carrier RNA. In addition, F1 showed STR profiles with less dropout alleles than F2. Moreover, dropout alleles appeared to be more frequent at longer storage time (Table 4, Figs. 6, 7).

Discussion

In forensic DNA typing, deteriorated and environmentally challenged samples can cause signal loss, peak imbalance, and allele dropout. However, when examining challenging samples DNA degradation isn't the only challenge to deal with.

The extraction of DNA from fabrics be challenging because of the co-extraction of PCR inhibitors which often present in the fabric (Belgrader et al., 1996). On the other hand, EZ1 showed to be more efficient than organic method in DNA extraction from bloodstained fabrics (black cotton and denim), as it overcomes the inhibitory effect of heme and dyes (like black dye and indigo), and the effect of sample storage time, especially when using carrier RNA as previously reported in many studies (Montpetit et al., 2005). Magnetic beads showed a great efficiency to extract more quantity of purified DNA samples and hence more efficient amplification may be contributed to the nature of silica-based matrices with specific DNA binding properties, since they are positively charged and have a high affinity for the DNA backbone negative charge.

As a result, the contaminants were eliminated with a series of washing steps followed by low ionic strength DNA elution ($pH \geq 7$) using TE buffer or sterile distilled water (Chacon Cortes & Griffiths, 2014). Moreover, in organic method, the product of proteinase K digestion of some heme-blood protein complex, was not completely extracted by organic solvents and remained in the DNA extract (Akane et al., 1994). In addition, organic method requires multiple tube changes which increase the possibility of contamination error with no complete removal of phenol from the extract and sample mishandling (Elkins, 2012).

In the presence or absence of carrier molecules, DNA extracted by robotic extractions (EZ1) was compared to DNA recovered using organic process. We found that DNA concentration of organic method was higher than that of EZ1 method, which was in harmony with several studies (Tsai & Olson, 1992; Akane et al., 1994; Valgren et al., 2008).The difference in yields could be attributable to the greater pre-treatment volume of phenol–chloroform-isoamyl alcohol used in the standard organic extraction procedure.

In the current study, poor DNA recovery from low-yield samples suggested that DNA binding to silica-coated magnetic particles is proportional to the concentration of nucleic acid molecules in the lysate up to a certain threshold, or that a fixed amount of DNA is lost due to non-specific binding to silica beads or container walls. These results led us to believe that the EZ1 could be improved by the addition of "carrier" RNA molecules for the extraction of DNA from evidence samples, which have been used with some success with various extraction methods to improve DNA recovery. This is in consistent with Ram Kishore et al. who reported that the DNA concentration extracted from EZ1 with carrier RNA was greater than that extracted with EZ1 without carrier RNA (Kishore et al., 2006).

On the other hand, DNA concentration extracted using organic method after addition of carrier RNA was lower when compared to that in absence of carrier RNA. This is in the contrary to the study of Kishore et.al., who stated that recovery of DNA was reproducibly improved when carrier RNA molecule was used during organic extraction (Kishore et al., 2006). This contradiction may be contributed to the role of carrier RNA that is believed to increase the partitioning coefficient, thus increasing the likelihood of the DNA binding to the silica monolith of the spin column and prevented it from being eluted during elution step which in return decreased the DNA concentration.

The concentration of DNA extracted via the two methods in the 1st day was greater than that extracted in the 14th day, may be because long time storage of the bloodstains at room temperature increase the susceptibility of DNA degradation, as DNA samples should be kept at−20 °C or lower for maintaining the integrity of DNA when going to be delayed (Hara et al., 2015). This is in harmony with the study of Anna et.al. who stated that quantification results from the extracted samples indicated that the DNA concentration is decreased over storage time; and DNA degradation is increased over storage time (Rahikainen et al., 2016).

However, STR Amplification needs higher amount of good quality and quantity of DNA input template to give a better STR allele calls for autosomal STR profiling. Although the DNA concentration of organic method is greater than that of EZ1, the RFU of STR alleles of the sample extracted by EZ1 was higher than that of organic.

Lower peaks height (RFU) using organic method prior to amplification than that produced by EZ1 may be due to the presence of inhibitors such as heme proteins and phenol which were not completely purified from DNA extract, in contrast to EZ1 at which most inhibitors are

Table 4 Allele dropouts from profiles using two extraction methods (EZ1 and organic), "with and without using carrier RNA" from two types of fabrics (F1 and F2), at two storage times (1st and 14th day)

PHR % less than 60		Fabric 1				Fabric 2			
		EZ		Manual		EZ		Manual	
		With carrier	Without carrier	With carrier	Without carrier	With carrier	Without carrier	With carrier	Without carrier
Volunteer 1	1st day						FGA(42%)	Amelognin(42%)	
								D19S433 (33%)	
	14th day			D7S820 (50%)			CSF1PO(40%), D8S1179 (50%)	D21S11 (50%)	Amelognin (50%) D21S11 (35%)
				CSF1PO (40%)			D5S818 (40%)		FGA (43%)
Volunteer 2	1st day		D19S433 (54%)	D2S1338 (22%)	D3S1358 (40%), D5S818 (50)				D13S317 (43%)
				D16S539 (40%)	D2S1338 (37%), CSF (49%)				
				D8S1179 (50%)	D18S51 (44%)				
	14th day	D8S1179 (49%)		D19S433 (42%)	D21S11 (50%), D2S1338 (52%)	D8S119 (49%)	D21S11 (0%)	D19S433 (42%)	Amel(0%), D21S11 (58%), TPOX
				D8S1179 (57%)	D13S317 (45%), FGA(52%)			D5S1179 (57%)	(0%), D19S433 (0%), CSF1PO(0%),13S317 (47%, D18S51 (0%), D21S11 (50%), D5S818 (52%), D135317 (45%)
Volunteer 3	1st day			Amelognin(46%)	D16S539 (44%), D8S1179		D21S11 (57%)	VWA(26%), D5S818 (59%)	D16S539 (28%), CSF1PO (33%)
				TH01 (49%)	(53%) D2S1338 (52%)		TPOX (58%)	D16S539 (29%), CSF1PO(24%), D13S317 (37%)	D13S317 (20%)
	14th day			TH01 (51%)	D16S539 (57%)	D5S818 (50%)			TH01 (52%), D16S539
				(30%)			D21S11 (50%)	D8S1179 (51%)	D13S317 (37%)
				TPOX(29%), D7S820 (38%)			TPOX(33%)		
Volunteer 4	1st day			Amel.(50%), D5S818 (29%)					
				D16S539 (51%), D8S1179 (0%)					
	14th day			D16S539 (59%)				TH01 (37%), D3S1358 (51%)	TH01 (53%), D3S1358 (56%), D16S539 (18%). CSF1PO(57%), D13S317 (0%), D18S51 (0%
				D18S51 (40%)				D21S11 (0%), D19S433 (0%)	
								D2S1338 (0%), D16S539 (32%) D18S51 (31%)	

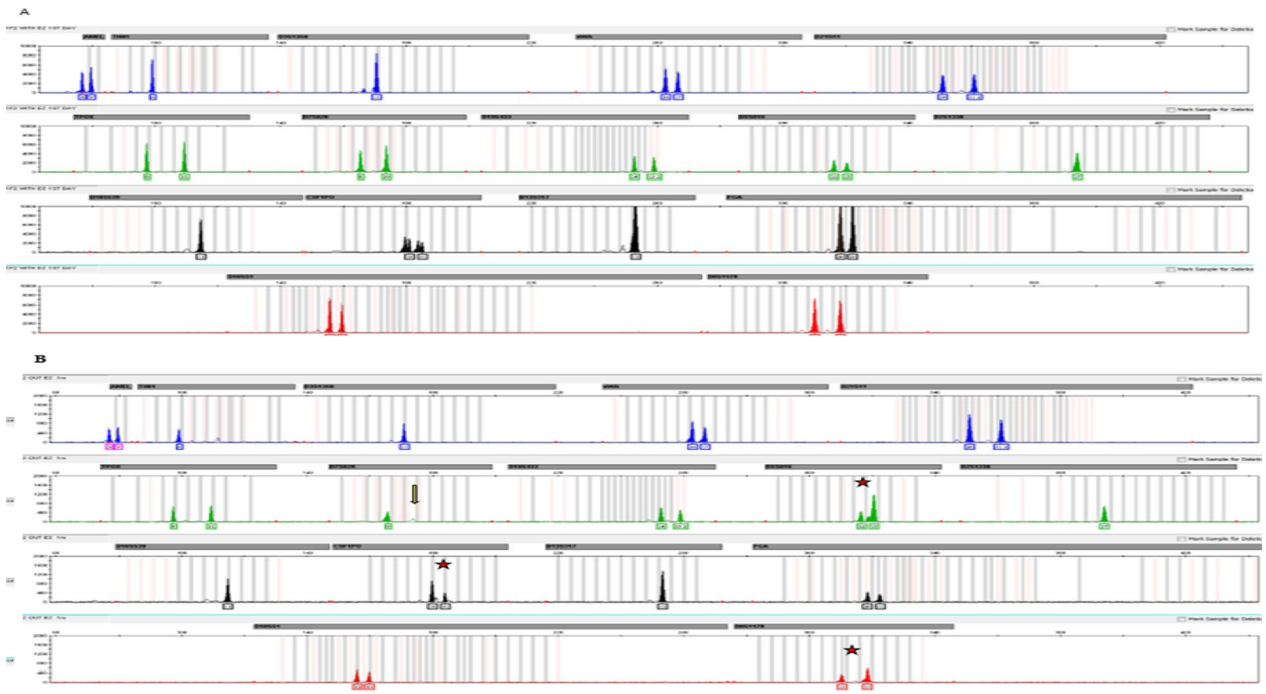

Fig. 6 Partial autosomal STR profiles of 200 µl blood on cotton using EZ1method after addition of carrier RNA (in the left) or without addition of carrier RNA (in the right) from four volunteers at 1st day storage) showed lower RFU,★ means peak imbalance less than 60%

removed. However, higher peak height (RFU) was produced using EZ1 with carrier RNA than that produced by using EZ1 without carrier RNA, this may be attributed to the role of carrier RNA which increased the DNA recovery.

The addition of carrier RNA seemed to improve STR genotyping for long lasting DNA samples. According to the results of several previous research, sample stored for long time produced partial STR profiles when extracted without carrier RNA, while a complete STR profile was produced from the same sample when carrier RNA was added. It has been noted that as the amount of DNA is decreased, the variation in heterozygote imbalance increases (Shaw et al., 2009; Sundari et al., 2021). This explained what we found at the 14th day storage time at which the DNA concentration decreased, and DNA degradation occurred, especially on using organic extraction which gave us an unreliable STR profiles with artifacts, dropout, heterozygote imbalanced peaks.

DNA analysis can be hindered by incomplete STR profiles formed by degraded DNA, due to allelic dropout, especially among larger STR loci. In addition, artifacts produced during the polymerase chain reaction (PCR) process may result in degradation of DNA, which were reported to reduce the reproducibility of DNA analysis due to stochastic effects (Butler, 2001).

Partial STR profiles produced especially in the 14th day were less in number using EZ1 as an extraction method than using organic method, probably may be due to more degraded DNA as a result of the presence of high level of PCR inhibitors resulted from less efficient DNA purification, that may reduce the activity of polymerase enzyme as well (textile dyes, indigo and chemicals used in organic). All these factors may participate in failure to amplify the larger STR loci in organic method (Bessetti, 2007).

Our data showed that F2 (denim) revealed partial STR profile when using EZ1 without carrier RNA, particularly at the 14th day of storage, although this was not the case for F1 (Black Cotton). The quality of the DNA profiles may have been influenced by the fabrics used, depending on the nature of the material, which correlates to previous findings in which clothing dyes (denim jeans dye) may contain polymerase inhibitors. Results indicated a loss of amplification efficiency that was possibly related to the quenching of the dye. Inhibited samples exhibit a partial profile with a specific pattern of locus dropout which is common in the pattern of degraded samples (Seah et al., 2004). Such inhibitors often produce electropherograms like that from degraded DNA (Latham & Miller, 2019; Taupin, 2013), so inhibited samples are thus erroneously believed to be degraded.

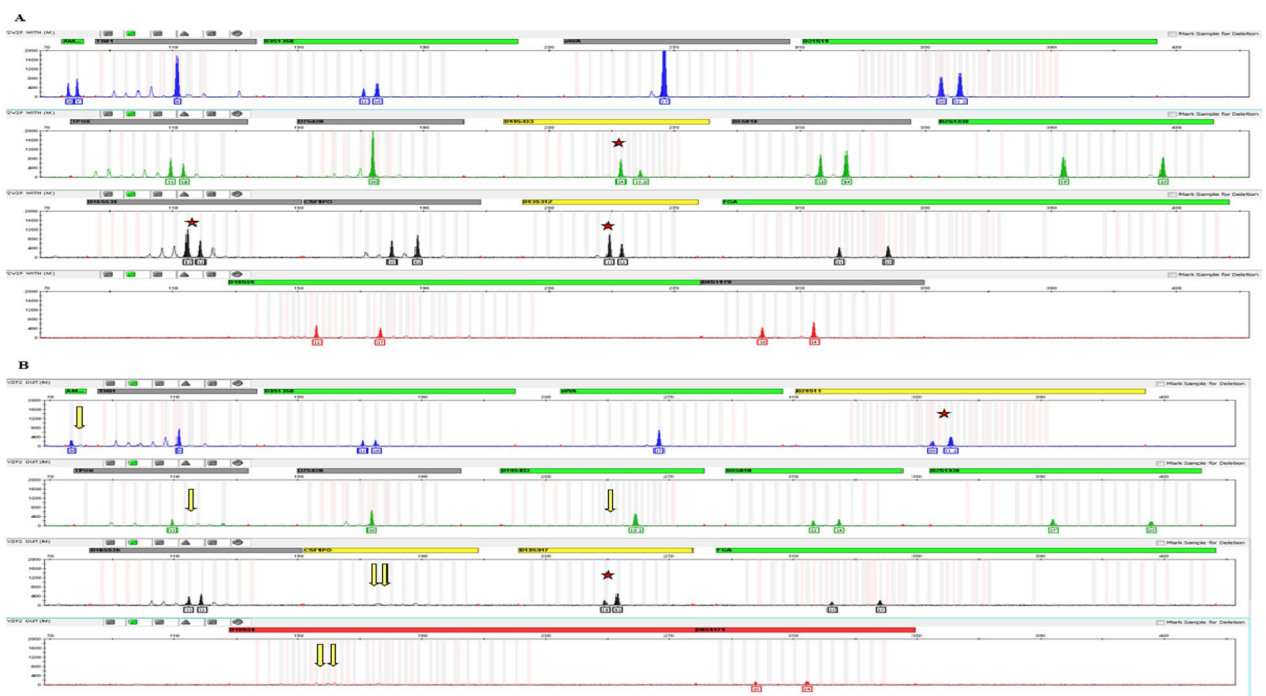

Fig. 7 Partial autosomal STR profiles of 200 µl blood on cotton using organic method after addition of carrier RNA(in the left) or without addition of carrier RNA(in the right) from four volunteers at 1st day storage) showed lower RFU, allele dropout, and locus dropout, and ★ means peak imbalance less than 60%

Regarding to the dropout phenomenon, which depends on the average amplicon size, the loci were evenly assigned into four size groups.: < 130 b.p (Amel., TH01, TPOX, D16S539), 130–220 (D3S1358, D7S820, D19S433, CSF1PO, D22S1045, D18S51), 200–300 bp (VWA, D19S433, D13S317), and > 300 bp (D21S11, FGA, D2S1338, D8S1179, D5S818) (Rahikainen et al., 2016). Moreover, DNA quantity and quality recovered were decreased over time to the point that it would be no longer possible to amplify large-amplicon targets, indicating that, during long storage, DNA integrity was affected. Accordingly, dropouts were reported in loci with larger amplicon sizes, such as, D5S818, D2S1338, D21S11, D13S317, D18S51 and FGA, and as well in loci with smaller amplicon sizes, such as Amelognin, D16S539, CSF, TH01, and TPOX.

Conclusions

We found that EZ1 with carrier RNA is preferred for bloodstain samples on fabrics with textile dyes (black dye or denim indigo), especially when a storage time passed over them, while organic is not preferred in that scenario. Phenol is considered a PCR inhibitor that affects the efficiency of amplification in addition to problematic dyed samples. Accordingly, fabric 2 (F2) was found to be more problematic than fabric 1 (F1) due to presence of challenging inhibitors (indigo dye) which lead us to turn to more advanced methods for DNA purification like EZ1 method other than using organic method with inhibitory components like phenol.

Small to medium-sized forensic laboratories can benefit from substantial time savings and more accurate case management by implementing EZ1 with DNA extractions in forensic casework without compromising sample quality. Organic extraction is less preferred when using fabrics with textile dyes like black and indigo even with addition of carrier RNA. Although the carrier RNA improved the STR profile quality, organic extraction is not preferred as well when the evidence samples stored for a long time but preferred when samples are available in small quantities. In addition, using carrier RNA during the organic method steps showed low concentration of the extracted DNA and no improvement of STR profiling.

Abbreviations

DNA: Deoxyribonucleic acid; PCR: Polymerase chain reaction; STR: Short tandem repeats; CODIS: (Combined DNA Index System); TE: Tris (hydroxymethyl) amino-methan-EDTA; µl: Microliter; ABI: Applied Biosystems Inc.; SWAGDAM: Scientific Working Group on DNA Analysis Methods; CE: Capillary electrophoresis; PHR: Heterozygote peak height ratios; RFU: Relative fluorescence unit; F1: Fabric one (black cotton); F2: Fabric two (denim.

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Author's contributions
HM has a major contribution in writing and revising the manuscript, SS made the practical part and analyzed and interpreted the STR data. MK performed the has a contributor in writing the manuscript. AA has revised the manuscript. All authors read and approved the final manuscript."

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Approval of the ethics committee was obtained from the Ethical Committee in the Ain Shams hospital, Egypt (No. FWA000017585). A written consent was taken from all the selected subjects.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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