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Evaluation of Selected DNA Extraction Method for Efficient Extraction under Various Conditions

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Abstract : DNA extraction is an important first step in DNA analysis for biomedical and forensic purposes. The DNA extracted from biological samples must be free from contaminants, such as protein and RNA. Although DNA may have to be extracted from different sources for forensic purposes such as saliva, semen, stains, hair and bones, blood samples are common objects of forensic analysis.DNA quality is a critical issue for most amplification-based analysis, since the DNA amplification is influenced by the presence of co-purified inhibitors from matrix or extraction reagents, which can decrease, subsequent Polymerase Chain Reaction (PCR) efficiency. DNA damage may also occur during the extraction procedure because of oxidation and enzymatic hydrolysis problems, associated with extraction buffers formulation and excessive mechanical shearing. This study therefore evaluated several methods in order to identify the most efficient extraction protocol for the production of DNA used in PCR technology. Blood samples were collected from 60 persons from Hila City-Iraq. These samples were used to simulate the different possible blood samples obtained for forensic medical purpose. The following simulations were created: 20 frozen blood samples, 40 dried blood stain samples on carpet.extraction of genomic DNA was attempted from the listed forms of blood samples by using four different methods: Promega purification kits, Favorgen purification kits, salting out methods, and two-step lysis assay. These methods were compared for the best extraction of DNA and were confirmed by molecular detection of β-globin gene using polymerase chain reaction (PCR) technique.
After extraction of DNA and electrophoresis by use agarose gel electrophoresis and measuring the concentration and purity of DNA by bio drop spectrophotometer. Purity was then confirmed by PCR. The results indicated that the best methods for genomic DNA isolation were from frozen, and dried stains on carpet using Promega purification kits.
Keywords: Forensic science, DNA extraction, Blood stain.

Introduction

Many different types of physical evidence are commonly submitted to forensic science laboratories for examination. Initially, evidence that was suitable for DNA analysis was limited to biological substances that contain nucleated cells, this limitation has been conquerin the last 5 years with the employment of mitochondrial DNA sequencing in the forensic field. Common biological specimens from which DNA has been successfully extracted and typed are as follows: bones, blood and bloodstains, semen and seminal stains, tissues, organs, teeth, hairs, fingernails, saliva, urine, and other biological fluids[1,2].The quantity of DNA that can be extracted from these common biological sources will vary Note that, in practice, crime scenes samples may contain considerably less usable DNA depending on environmental conditions. DNA has been extracted from

other sources, such as gastric fluids and fecal stains. However, it can be difficult to generate a DNA profile from these sources in case samples due to significant degradation[2].

Many factors affect the ability to obtain a DNA profile, there are several factors, 1. Sample quantity, the sensitivity of polymerase chain reaction- based (PCR) DNA typing methods is noteworthy, but still limited. 2. Sample degradation, prolonged exposure of even a large blood stain to the environment or to bacterial contamination can degrade the DNA and render it unsuitable for further analysis. 3. Sample purity, most DNA typing methods are robust, and dirt, grease, some dyes in fabrics, and other substances can seriously compromise the DNA typing process, environmental insults will not change DNA allele "A" into allele "B", but they can adversely affect the ability of the scientist to obtain a complete DNA profile from the sample[3].

Blood is the most common body fluid encountered at crime scenes,it is a very complicated liquid tissue and serves as the transporting medium for all the substances in the body[4].

Blood identification is central to many homicide investigations and is also useful in cases involving aggravated assault, sexual assault, and burglary, The evaluation of blood evidence can be crucial to substantiate a complainant's or suspect's account of alleged events, the presence of blood on evidentiary items can be critical in establishing guilt or innocence during criminal proceedings, the analysis of blood evidence can be important not only in establishing which individual might have been bleeding, but also in the manner in which blood was deposited[5].

Bloodstain pattern analysis, BPA, on hard surfaces (such as walls, tables, appliances, hardwood floors, etc.) has grown into a science-based investigative tool that can help determine scenarios that are consistent with or counter to the events described by witnesses or suspects, at the vast majority of crime scenes involving a bloodletting event, textiles are present as apparel, household textiles (sheets, towels), upholstery, carpets, and so forth. Yet, the science of BPA is not able to render the same level of confidence in the analysis as on hard surfaces due to the complex structure of textiles and their ability to wick liquids[6].

Materials and methods

subject

Sixty human blood samples(20 from frozen blood & 40 from dry stain on carpet)were collected from persons in the Hila City- Iraq, the study was conducted during period from19/6-8/9/2016. Carpet samples were (20) samples recovered after 2 hours and (20) samples recovered after 3 hours.DNA extraction conducted by using two extraction kits (Promega kit &Favorgenkit) and two manual methods (salting out & two lysis step assay).

Procedures

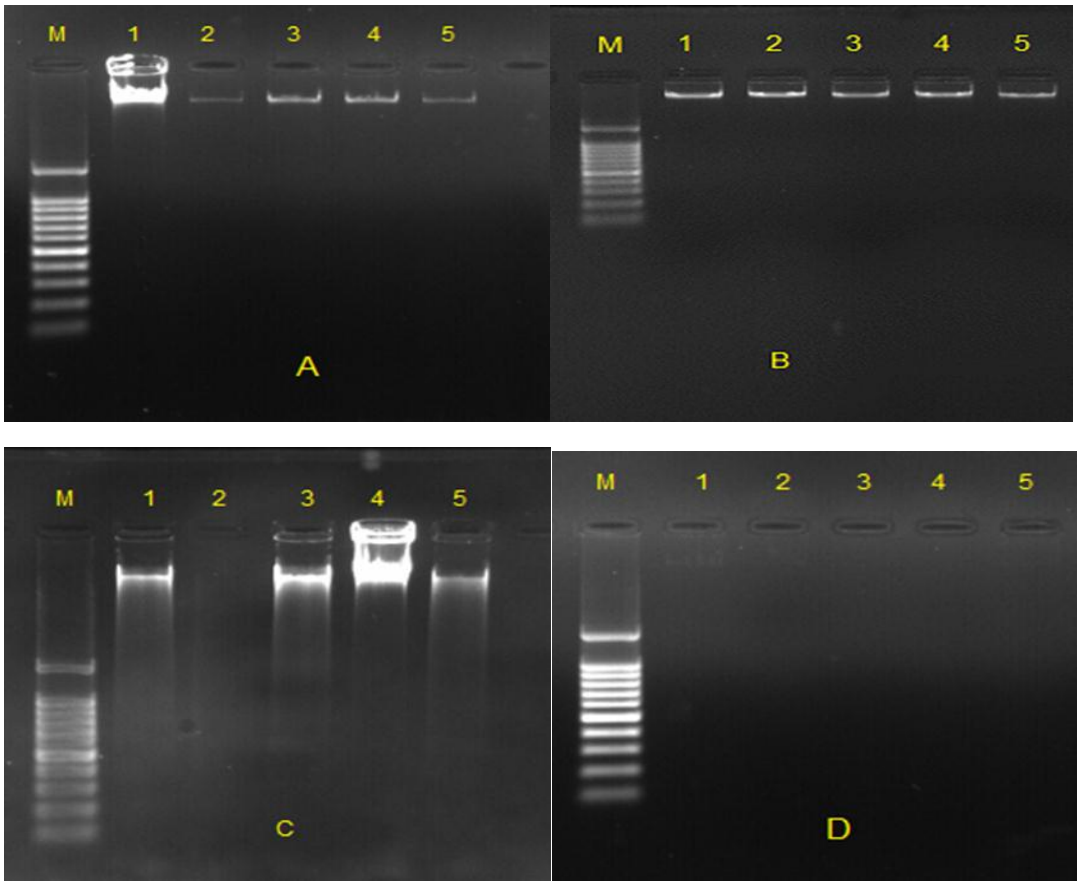
DNA extraction from frozen blood conducted by Following the relativeprotocol of(Promega kit &Favorgenkit), in DNA extraction from blood stain on carpet, small carpet cutoffs were cut by using sterilized scissors then adding RBC lysis buffer vortex for 1 min and leave it in room temperature for 20 min, then carpets cutoffswere discarded and complete the relative protocol of DNA extraction for each method.

DNA extraction by salting out method adopted by the protocol which mentioned by[7] while two lysis step assay dependenton [8] protocol, the same previous context of DNA extraction from blood stain recovered from carpet in two different time was followedin manual extraction.

Results

The results showed a fluctuation in the level of purity and concentration of extracted DNA among these four different extraction methods. The best method of DNA extraction for frozen and dried spots on carpet samples was Promega kit, DNA bands were very clear in gel electrophoresis figure (1) A, figure (2) A, and figure (3) A. The concentration and purity of DNA extracted by Promega kit was good compared with other

methods. The DNA values in frozen samples ranged from 6 to 26µg/ml for concentration and from 1.4 to 1.9for purity table (1), different results were obtained for DNA extracted from dried stains on carpet. The present results showed a concentration of (9-14 µg/ml) and (6-25µg/ml) and the purity were (9-14) and (1.5-2) after two and three hours respectively, table (2) and table (3).

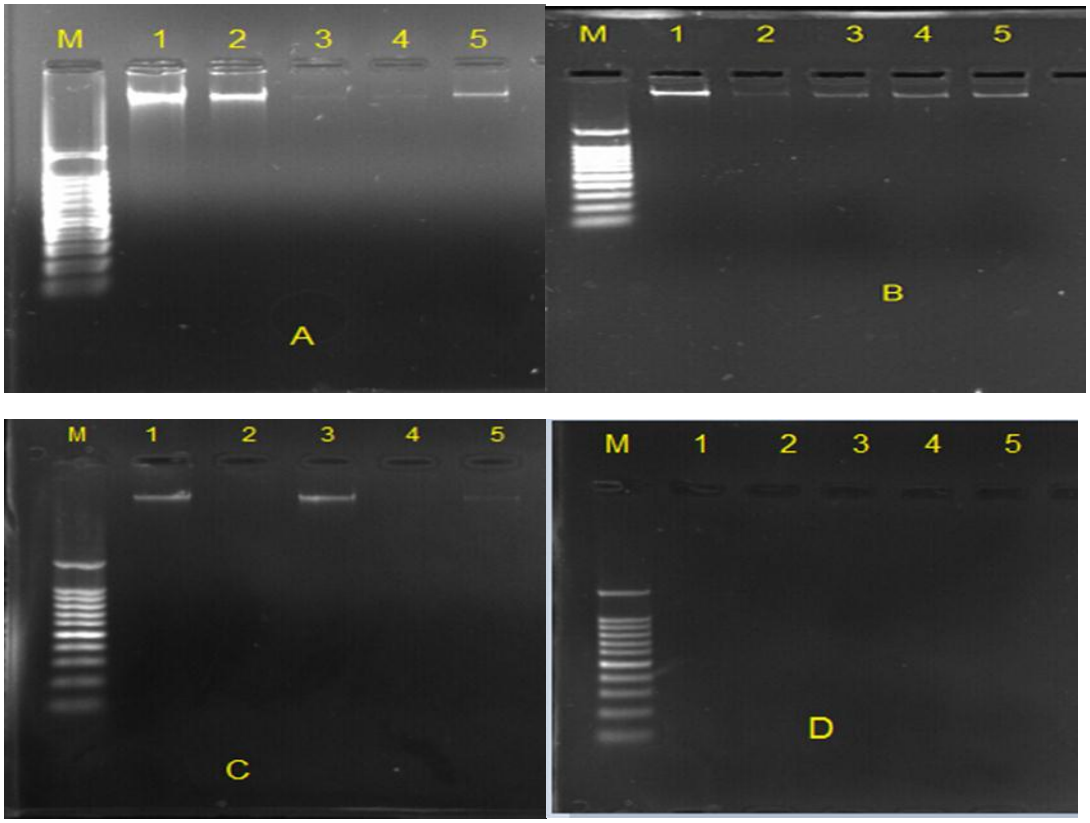


Fig(1): Gel electrophoresis of extracted DNA from 5 samples of human frozen blood A: Promega kit B: Favorgen kit C: salting out method D: two-step lysis assay, at 70 volt for 1 hours, line M:100bp DNA marker.

Table (1): purification and concentration of extracted DNA from human frozen blood using four different methods.

Human Frozen Blood Sample	**Promega Kit**		**Favorgene Kit**		**Salting Out Method**		**Two – Step Lysis Assay**	
	Conc. (µg/ml)	Puri.	Conc. (µg/ml)	Puri.	Conc. (µg/ml)	Puri.	Conc. (µg/ml)	Puri.
1	26	1.9	6	1.4	35	1	0	0
2	12	1.4	6	1.5	85	1	0	0
3	6	1.8	10	1.4	116	0.9	0	0
4	6	1.8	57	1.2	87	0.9	0	0
5	11	1.8	35	1.5	46	0.8	0	0

- **Conc.: Concentration.**
- **Puri.: Purification**

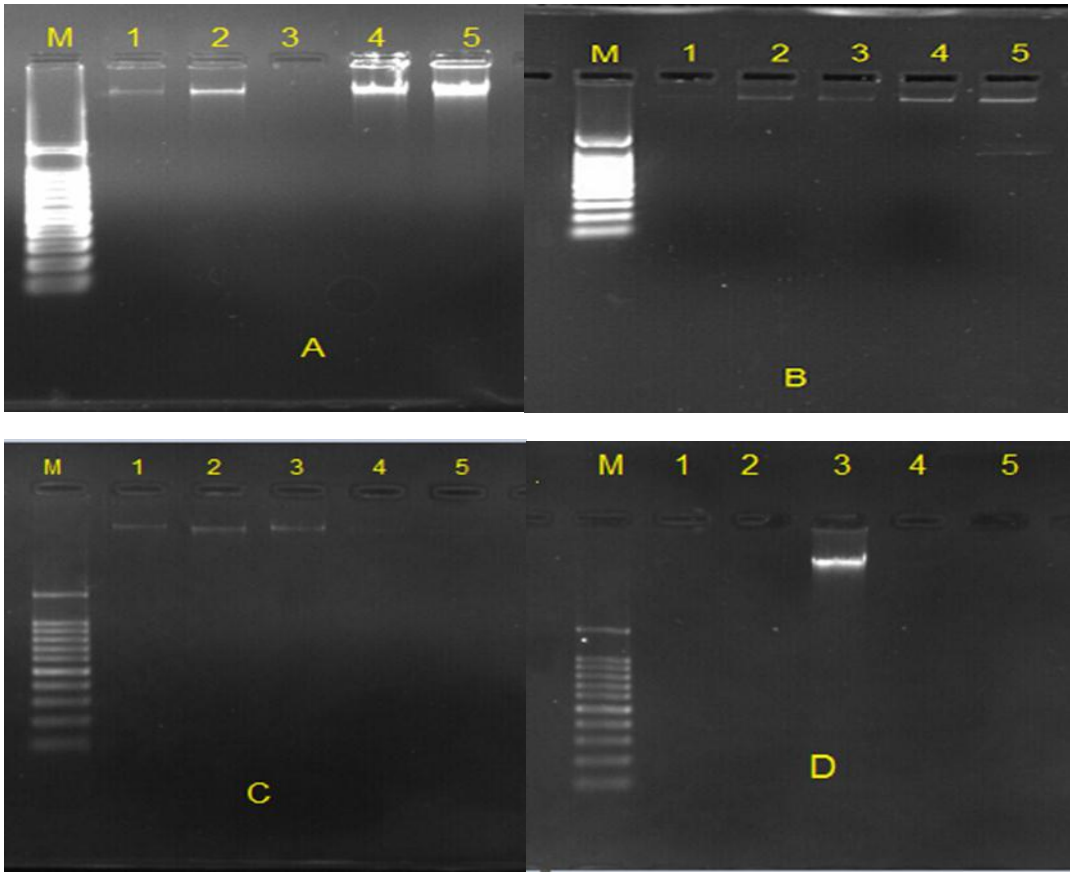


Fig(2) : Gel electrophoresis of extracted DNA from 5 samples of human blood spots on carpet after 2 hours A: promega kit B: favorgen kit C: salting out method D: two-step lysis assay, at 70 volt for 1 hours, line M:100bp DNA marker.

Table (2) : purification and concentration of extracted DNA from human dried blood spots on carpet after 2 hours using four different methods.

Human Dried Blood Sample	**Promega Kit**		**Favorgene Kit**		**Salting Out Method**		**Two – Step Lysis Assay**	
	Conc. (µg/ml)	Puri.	Conc. (g/ml)µ	Puri.	Conc. (µg/ml)	Puri.	Conc. (µg/ml)	Puri.
1	13	1.6	8	1.5	206	1.4	0	0
2	9	1.7	1.3	0.6	122	1.2	0	0
3	10	1.6	6	1.6	8	1.3	0	0
4	10	1.6	4	1.3	84	1.4	0	0
5	14	1.8	12	1.5	31	1.1	0	0

- **Conc.: Concentration.**
- **Puri.: Purification .**



Fig(3) : Gel electrophoresis of extracted DNA from 5 samples of human blood spots on carpet after 3 hours A: promega kit B: favorgen kit C: salting out method D: two-step lysis assay, at 70 volt for 1 hours, line M:100bp DNA marker.

Table (3) : purification and concentration of extracted DNA from human dried blood spots on carpet after 3 hours using four different methods.

Human Dried Blood Sample	**Promega Kit**		**Favorgene Kit**		**Salting Out Method**		**Two – Step Lysis Assay**	
	Conc. (µg/ml)	Puri.	Conc. (µg/ml)	Puri.	Conc. (µg/ml)	Puri.	Conc. (µg/ml)	Puri.
1	11	1.5	1.85	0.6	12	1.3	0	0
2	6	1.8	2.5	1.6	17	1.2	0	0
3	25	2	0.4	0.8	41	1.1	731	1
4	6	1.6	0.85	0.9	24	1.3	0	0
5	15	1.7	0.12	1	33	1.1	0	0

- **Conc.: Concentration.**
- **Puri.: Purification**

PCR detection

Polymerase chain reaction (PCR) was used to detect β-globin gene which found originally in blood, sixteen samples of extracted DNA were chosen randomly to detect this gene. PCR product for this gene approximate 500bp in size, this step was conducted to ensure that the extracted DNA is pure, the results of PCR product was as shown below:

Only the DNA samples which extracted by promega kit gave a product and the band was very clear in gel electrophoresis, figure (4) A, Line 1-3, while other sample didn't gave productor the band were like smear, figure (4) A, Line 4-8 and B, Line 1-8.

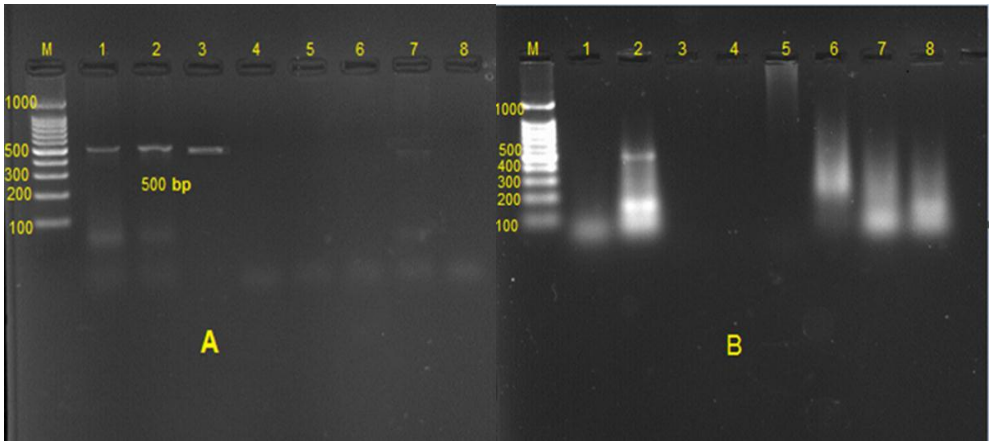


Figure (4) : Electrophoresis of PCR products of β-globulin gene on 2% agarose at 70 volt for one and half hour, line M: 100bp DNA marker.

Discussion

The present results are similar with the previous findings that showed the superior extraction ability of Promega kits compared with other methods. [9] refers that Promega kit is large-volume purification systems for manual and automated purification of genomic DNA from blood.[10]compared a combination of manual and automated extraction methods for DNA extraction from whole blood samples and finally they modified the Wizard SV 96 Genomic DNA Purification Kit (Promega Corporation), combining it with magnetic separation using the Mag NA pure purification method. This modification gave higher concentration and purity of extracted DNA than using Promega kit alone. Other studies showed that kits are more efficient in recovering DNA than manual methods,[11] compared the efficiency of silica (QIAamp DNA Mini Kit), Chelex and Phenol-Chloroform based techniques to recover DNA from different categories of samples including blood and saliva on cotton swabs, muscles, cigarette butts, saliva on foods and epidermal cells on clothes. They found that the efficiency of the QIAamp system was better than those of Chelex or Phenol-Chloroform techniques.

Although the concentration of extracted DNA in salting out method and Favorgene kit was high but the purity levels were unacceptable in some samples as shown in table (1), table (2) and table (3), perhaps the reason attributed with the extraction technique and contamination by chemicals in manual extraction, this agreed with[8] results who refers that a number of chemicals such as phenol, salts, polyamines, polysaccharides are used that may still be contaminated with the DNA solution.

The reasons for PCR results could be attributed to the quality of samples, DNA samples which extracted by Promega kit were more pure than others, this affected on PCR product that agreed with[12] who referred that the choice of method used for nucleic acid extraction will be a major determinant on the final quantification, nucleic acid extraction efficiencies vary considerably between different methods and the final nucleic acid yield is dependent on both the method used and the type of environmental sample being studied.[13] mentioned that PCR amplification is highly dependent on the size distribution and quality of the extracted DNA, which is why amplifications using the same amount of DNA can produce different results.

On the other hand, manual extraction significantly influenced on the quality of extracted DNA specially the chemical material that use in this methods which agreed with[14-20] who mentioned that several drawbacks bioengineering are associated with phenol/chloroformbased extraction techniques such as working with hazardous chemicals and many repetitive steps resulting often in phenol contamination of the extract and (partial) inhibition of subsequent PCR, as well as poor extraction efficiency.

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