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Comparison of Different DNA Extraction Methods for Forensic Samples

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Abstract
DNA analyses are important for different types of research in biomedical and forensic science and DNA isolation is an often required step. Because different DNA isolation kits are constantly becoming available, it is useful to compare their efficacies. This study compared kits of the QIAamp DNA Investigator (Qiagen), ZR Genomic DNA (Zymo Research) and AccuPrep Genomic DNA (Bioneer). Spectrophotometry showed the highest yield of DNA from 200 µl of blood was obtained by the Qiagen kit (5.6 µg) followed by the Bioneer kit (2.74 µg) and Zymo kit (2.39 µg). For purity measured by spectrophotometric A260/A280 nm ratio, DNA isolated by the Qiagen was the most pure and had a ratio of 1.85, closer to the ideal ratio than DNA isolated by Zymo (2.0) and Bioneer (1.03) ratios. Costs of isolation were $3.60/sample for Qiagen, $1.50/sample for Bionner and $0.72 for Zymo kits. The Zymo test took 25 minutes to isolate DNA, the Bioneer kit 35 minutes and the Qiagen kit 50 minutes. These findings may help laboratories select DNA kits that suit their purposes.
Keywords: Forensic science, DNA, extraction, electrophoresis, spectrophotometry, spin-column

1.1. Introduction

DNA isolation is an important first step in DNA analysis for biomedical and forensic purposes (Comey, 1994). The DNA isolated from biological samples must be free from contaminants, such as protein and RNA (Klein et al, 2002). Although DNA may have to be isolated from different sources for forensic purposes such as saliva, semen, stains, hair and bones, blood samples are common objects of forensic analysis. The methods for genomic DNA isolation from whole blood usually involve disruption and lysis of the blood sample by detergent solubilization of membrane lipids and proteolytic removal of proteins. A final step may involve precipitation of the DNA with ethanol or isopropanol or its adsorption to a filter or matrix.

Different methods for the isolation of genomic DNA may thus rely on different physical processes (Butler, 2005; Rechsteiner, 2009; Nielson et al, 2008; Saiyed et al, 2008; Nishiguchi et al, 2002).

Organic and non-organic extraction, Chelex removal of cations and FTA paper and solid-phase extraction techniques are commonly used for DNA isolation in forensic DNA laboratories (Walsh, 1991; Greenspan, 2004). The organic extraction process is a well established technique which removes and precipitates proteins from DNA by using phenol-chloroform. DNA is isolated from the aqueous layer by precipitation by ethanol in the presence of salt. However, this organic extraction method is time consuming.

Chelex extraction isolates DNA by employing an ion exchange resin that binds divalent cations. Isolation through the use of Chelex denatures double stranded DNA and yields single stranded DNA. The DNA extracted by using Chelex approach can be used for PCR-based typing (Butler, 2005).

The FTA™ paper method involves adding blood to special filter paper and drying the stain. The DNA on the FTA™ can be stored at room temperature and eluted by a buffer (Lorente, 2004; Butler, 2005). Solid-phase extraction methods for DNA purification exploit selective absorption of DNA from lysates to silica filters or magnetic beads. Recovery of DNA from these absorbents is accomplished after washing the supports by elution with select buffers.

Castella et al. compared the efficiency of silica (QIAamp DNA Mini Kit), Chelex and Phenol-Chloroform based techniques to recover DNA from different categories of samples, 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. Satia-Aboutia et al. compared the DNA yield, quality, and associated costs of buccal cell DNA collected using cytobrushes and mouth rinsing in self-administered procedures. The DNA samples they used were extracted by using QIAamp mini kits (Qiagen Inc.). It was found that total DNA yield was 30% higher from the mouthwash than the cytobrush buccal cell

collection procedure.
Certain DNA extraction technologies for forensic purposes were also reviewed in one study that reported the usefulness of automation and kits (Rechsteiner, 2006).
Previous studies have provided information on useful DNA sample processing and analysis methodologies. However, the comparison of the efficacy of DNA isolation among AccuPrep Genomic DNA Extraction Kit, ZR Genomic DNA Kit, and QIAamp DNA Investigator Kits has not been reported.
New commercial kits for DNA extraction isolation are continually being made available. These kits may vary in different characteristics that are of importance to analysts such as time for processing, yield, purity and integrity of DNA, cost and simplicity of process. Consequently, it can be useful to measure such parameters in comparison of different commercially available kits for DNA isolation. DNA analysts and educational institutions would thus have more criteria for the selection of kits for particular needs.
The objectives of the present study were to compare the characteristics for three kits for the isolation of DNA from blood. Parameters examined were those of purity, integrity, yield, time to complete isolation and cost.
Some reported DNA isolation techniques are summarized in Table 1.

1.2. Methods and Materials

Three kits were evaluated for the isolation of DNA from 3 different human whole blood samples. The kits evaluated included the AccuPrep Genomic DNA Extraction Kit (Bioneer), ZR Genomic DNA Kit (Zymo Research), and QIAamp DNA Investigator Kit (Qiagen). DNA extraction procedures were performed by following the manufacturer's instructions. Each extraction was performed three times. Samples were stored at 4°C until analysis.
The AccuPrep Genomic DNA Extraction Kit was purchased from Bioneer Corporation. The procedure was as follows: 20µl of Proteinase K was added to a 1.5 ml micro centrifuge tube followed by 200µl of whole blood and 200 µl of Binding buffer. The sample was mixed immediately by vortex mixer. After 10 minute incubation at 60°C, 100 µl of Isopropanol was added to the sample and mixing performed by pipetting. The lysate was transferred to a filter column in a micro centrifuge tube. After centrifugation at 8,000 rpm for 1 minute, the filter was washed with Wash buffer 1 and then Wash buffer 2. Finally, the genomic DNA was eluted by use of 200 µl elution buffer.
According to the manufacturer, the AccuPrep Genomic DNA Extraction Kit can isolate an average of 6 µg of total DNA from different sources, such as 200µl of whole blood, 5 x 10^6 leukocytes and 25-30 mg mammalian tissues.
The ZR Genomic DNA Kit was obtained from Zymo Research Company. The procedure started with the addition of 50µl whole blood to a 1.5 ml micro centrifuge tube followed by 250 µl of Genomic Lysis Buffer and 10 µl Zymobeads. The sample was mixed and incubated for 2 minutes at room temperature. After centrifugation at 5,000 rpm for 1 minute, 250 µl Lysis Buffer was added to the pellet which was resuspended and re-centrifuged at 5,000 rpm for 1 minute. The Zymobeads-DNA complex was washed twice by centrifugation with 500 µl Wash Buffer. The pellet of beads was mixed with 35 µl of Elution Buffer and incubated at 65 °C for 5 minutes. The eluted DNA was collected after centrifugation of the beads for 1 minute at 10,000 rpm.
The QiAamp DNA Investigator Kit was purchased from Qiagen Company. Isolation of DNA began with the addition of 10 µl of proteinase K to 100 µl whole blood and 100 µl Buffer AL. After 10 minutes of incubation at 56 °C, 50µl ethanol was added. The mixture was incubated for 3 minutes at room temperature and then transferred to a QIAamp MinElute column and centrifuged at 8,000 rpm for 1 minute. The column was washed with 500 µl W1 buffer and also with 700 µl W2 buffer by centrifugations at 8,000 rpm for 1 minute each. The column was washed with 700 µl ethanol. The filter-bound DNA was eluted with 50 µl of ATE buffer.
Yields and quality of the DNA isolated by the three kits were measured by use of a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific). DNA quality was assessed using the absorbance ratio at 260:280 nm. DNA and protein absorb differently at these wavelengths. An A260/A280 $\geq$ 1.8 is generally accepted as "pure" for DNA; a ratio of $\geq$ 2.0 indicates that the preparation is contaminated with RNA; a ratio of $\leq$ 1.8 indicates a possible contamination with proteins or other contaminants that absorb strongly at or near 280 nm.
The quality of the isolated DNA samples were also analyzed by electrophoresis on 0.8% agarose (wt/vol) gels using Tris, Borate EDTA pH 8.3 buffer and ethidium bromide staining. Gel imaging was done by using a Fluor Chem Q Analyzer from Alpha Innotech Company.
The cost per extraction was calculated for the commercially availability kits by dividing the cost by the number of extractions that could be performed with the kit.
The processing times required for manual manipulation of tubes, reagents, and samples were tabulated separately from non-manipulation time (e.g., incubation, drying, etc.).

1.3. Results

The AccuPreP Genomic DNA Extraction Kit was used to isolate DNA from three blood samples and the yields were measured by uv-vis spectroscopy. The results in Table 2 showed that the DNA concentrations were 12.3 ng/µl, 12.5 ng/µl, and 16.2 ng/µl. The average concentration was 13.7 ng/µl. The average total DNA yield of in final volume of 200 µl from a starting volume of 200µl whole blood was 2740 ng (Table 4).

The results of uv-vis spectrophotometry for DNA isolated with the ZR Genomic DNA Extraction Kit are shown in Table 3. The concentrations of three preparations of DNA were 15 ng/µl, 25.1 ng/µl, and 11.2 ng/µl. The average concentration was 17.1 ng/µl. The total DNA yield in 35 µl from a starting volume of 50 µl whole blood was 598.5 ng. This would represent a yield of 2394 ng DNA from 200µl whole blood (Figure 4).

The results from uv-vis spectrophotometry of DNA isolated with the QIAamp DNA Investigator Kit are presented in Table 2. The results show that the concentrations of DNA isolated from three blood samples were 52 ng/µl, 65 ng/µl, and 49.6 ng/µl. The average concentration of the isolated DNA was 55.5 ng/µl. The total DNA yield in a final volume of 50 µl starting from 100 µl whole blood was 2775 ng This would represent a yield of 5550 ng DNA from 200µl whole blood (Figure 4).

DNA Quality

The results in Table 3 for DNA isolated by use of the Bioneer Accuprep Genomic NA Extraction kit showed that the ratio of sample absorbance at 260 nm and 280 nm were: 1.10, 0.95, and 1.03. The average A260/280 was 1.03. The results in Table 3 for DNA isolated using the ZR Genomic DNA Extraction Kit showed A260/A280 nm ratios of sample absorbance were 1.93, 2.01, and 2.07. The average ratio of A260/280 was 2.00. For DNA isolated using the QIAamp DNA Investigator Kit, the ratios of sample absorbance at 260 nm and 280 nm were 1.83, 1.88, and 1.84, respectively (Table 3). The average ratio of A260/280 absorbance was 1.85.

DNA isolated by using the AccuPrep Genomic DNA Extraction Kit was analyzed by agarose gel electrophoresis and visualized by ethidium bromide staining. The results in Figure 1 were compatible with the isolated DNA being of high quality in all the samples.

Similarly, agarose gel electrophoresis analysis of DNA isolated using ZR Genomic DNA showed the patterns consistent with the presence of intact DNA in Figure 2.

The results of agarose gel electrophoresis of DNA isolated by using QIAamp DNA Investigator Kit are shown in Figure 3. The patterns were consistent with a high quality DNA preparation and the staining of bands was more intense in accordance with the higher concentrations of DNA isolated by this procedure.

1.4. Discussion

This study performed a comparison of the performance of three commercial kits for DNA isolation from human blood. Table 4 summarizes the comparison of DNA concentration, DNA purity, DNA yield, cost per sample, and the processing time for each kit.

It was observed that the DNA obtained by the QIAamp DNA Investigator Kit produced the highest concentration, an average of 55.5 µg / µl, while the other two kits only yielded 13.7 µg / µl (Accuprep) and 17.1 µg / µl (ZR Genomic DNA) DNA from 200 µl of human blood. The significance of these results is that large differences were seen in the DNA concentration between the three kits used.

Measurement of DNA purity by using the A260:280 ratio showed an average DNA ratio of 1.85 for the DNA isolated with QIAamp DNA Investigator Kit, and a ratio of 2.0 for the DNA isolated with ZR Genomic DNA extraction Kit. A ratio of 1.03 only was obtained for indicated that the DNA isolated by the AccuPrep Genomic DNA Extraction Kit which indicated the presence of contaminants that absorb strongly at or near 280 nm. Based on this criterion, the Qiagen QIAamp kit produced the highest purity DNA.

It was found that the QIAamp DNA Investigator Kit produced the highest DNA yields among these three kits. About 5.6 µg of DNA was isolated in 200 µl of eluent obtained from a starting volume of 200 µl of whole blood using QIAamp DNA Investigator Kit, while the other kits only yielded 2.39 µg and 2.74 µg of DNA from 200 µl of whole blood. Thus, the QIAamp DNA Investigator Kit that yielded about twice as DNA as the other two kits used.

The cost of isolating DNA from human blood was calculated based on actual charges for the commercially available kits by dividing the cost of the kit by the number of extractions that could be performed with the kit. The most economical method was the **ZR Genomic DNA Kit™** at just $ 0.72 per test, while the QIAamp DNA Investigator Kit, priced at $3.60 per test, was considerably more expensive than the other kits.

Processing time varied considerably between the kits, ranging from as little as 25 min for completion of the ZR Genomic DNA Kit to as long as 50 min for the QIAamp DNA Investigator Kit (Table 4).

In summary, the results of this study demonstrate that the QIAamp DNA Investigator Kit performed better than two other commercial methods evaluated for the extraction of DNA from human blood and it yielded purer and larger amounts of DNA than two other kits. However, the QIAamp DNA Investigator Kit was also the most expensive and time consuming method of these three kits. The ZR Genomic DNA Kit was the cheapest and

fastest method of the three DNA isolation kits tested. It only required about 20 to 25 minutes completing an isolation and it only cost $0.72 per test.
QIAamp columns performed better than the other commercial methods evaluated for isolation of DNA but also involved double the time and the money for DNA isolation.
The ZR Genomic DNA Kit could be of advantage in laboratories to obtain reasonably pure DNA where speed of sample processing time is a paramount need.

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Table 1.
Characteristics of the three DNA extraction kits evaluated in this study

Method	Name	Vendor	Catalog no.	Main features
1	AccuPrep Genomic DNA Extraction Kit	Bioneer	K-3032	proteinase K digestion, DNA binds to glass fibers, chaotrope present
2	ZR Genomic DNA Kit	Zymo Research	D3004	Genomic Lysis Buffer, Zymobeads bind DNA, water elution.
3	QIAamp DNA Investigator Kit	Qiagen	56504	proteinase K digestion, DNA binds silica-gel membrane

Summary information on the three DNA isolation kits were derived from the manufacturer's manuals.

Table 2.
QIAmp gives the highest yield of DNA among three different DNA isolation methods

	BioNeer	Zymo	QIAamp
Sample 1 (ng/µl)	12.3	15.0	52.0
Sample 2 (ng/µl)	12.5	25.1	65.0
Sample 3 (ng/µl)	16.2	11.2	49.6
Mean	13.7	17.1	55.5
Median	12.5	15.0	52.0
SEM	2.2	7.2	8.3

A Nanodrop micro-volume uv-vis spectrophotometer was used to determine the concentrations of DNA isolated from 3 different human blood samples by use of kits from Bioneer, Zymo Research and Qiagen.

Table 3.
QIAmp method yields the highest purity of DNA isolated by use of three different kits

	BioNeer	Zymo	QIAamp
Sample 1 (ng/µl)	1.10	1.93	1.83
Sample 2 (ng/µl)	0.95	2.01	1.88
Sample 3 (ng/µl)	1.03	2.07	1.84
Mean	1.03	2.00	1.85
Median	1.03	2.01	1.84
SEM	0.075	0.070	0.026

The A260/A280 ratios were determined as an index of purity for DNA isolated from three different human blood samples by the use of three different methods. Pure DNA has ratio of 1.8.

Table 4.
Cost-benefit comparison of DNA isolation kits

Extraction kit	DNA concentration (ng/µl)	DNA purity A260/A280nm ratio	DNA yield (µg) (200µl blood)	Cost ($)	Time (min)
AccuPrep Genomic DNA Extraction Kit	13.7	1.03	2.740	1.50	35
ZR Genomic DNA Kit	17.1	2.00	2.394	0.72	25
QIAamp DNA Investigator Kit	55.5	1.85	5.550	3.60	50

Average DNA concentrations and purity were determined by micro-volume uv-vis spectrophotometry for DNA preparations from 3 blood samples. The time used for preparation was calculated by adding the times involved in the different steps in the procedures. The costs per sample were calculated by dividing the costs of the kits by the number of samples that the kits could purify.

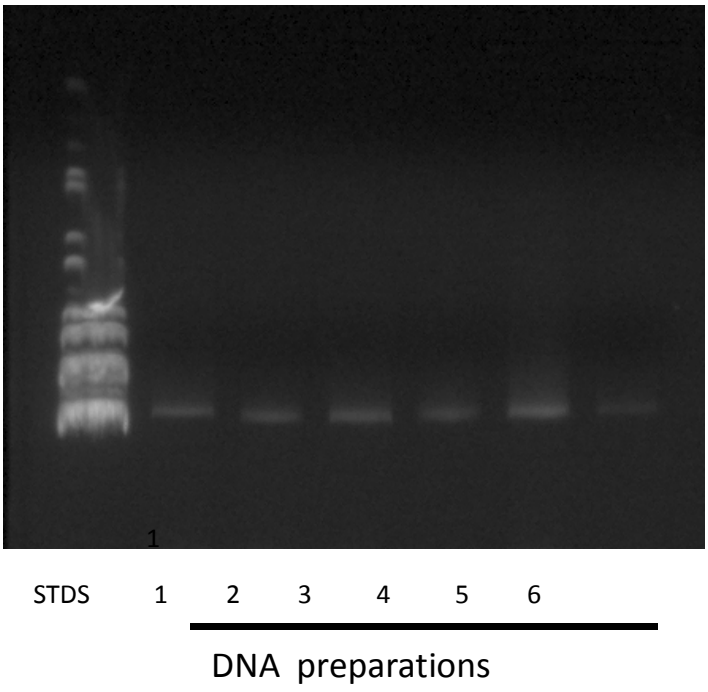
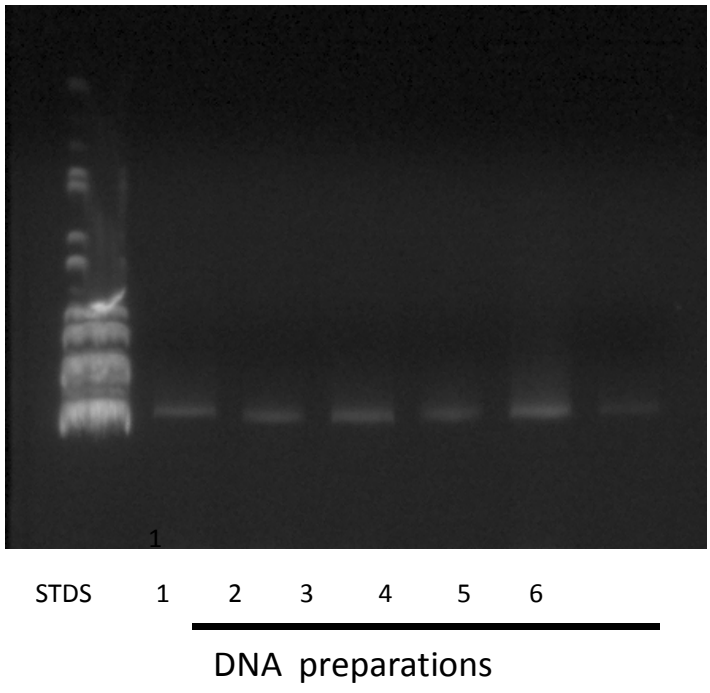


Figure 1. Electrophoresis shows intact DNA is isolated with Qiagen QIAamp Investigator Kit
DNA isolated from three human blood samples were separated on a 0.8% agarose gel and stained with ethidium bromide. Left-right: STDS-wide range DNA size standards (1) 1st blood sample 1 DNA (2) 2nd blood sample 1 DNA (3) 1st blood sample 2 DNA (4) 2nd blood sample 2 DNA (5) 2nd blood sample 3 DNA (6) 2nd blood

sample 3 DNA



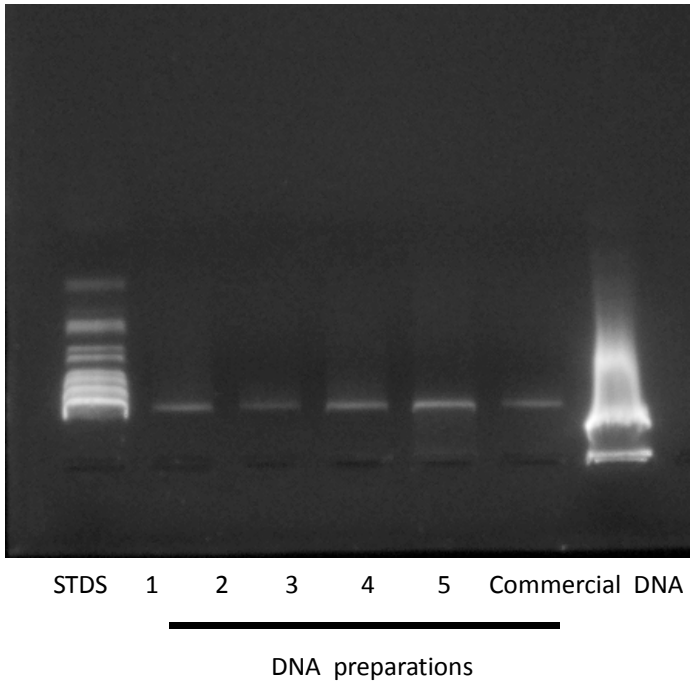


Figure 2. Electrophoresis shows intact DNA is isolated with ZR Genomic DNA Extraction Kit

DNA isolated from three human blood samples were separated on a 0.8% agarose gel and stained with ethidium bromide. Left-right: STDS-wide range DNA size standards (1) 1st blood sample 1 DNA (2) 2nd blood sample 1 DNA (3) 1st blood sample 2 DNA (4) 2nd blood sample 2 DNA (5) 2nd blood sample 3 DNA (6) 2nd blood sample 3 DNA

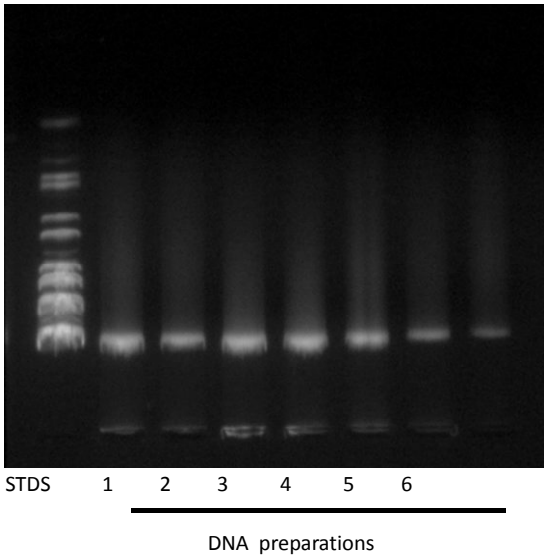


Figure 3. Electrophoresis shows intact DNA is isolated with Qiagen QIAamp DNA Investigator Kit.

DNA isolated from three human blood samples were separated on a 0.8% agarose gel and stained with ethidium bromide. Left-right: STDS-wide range DNA size standards (1) 1st blood sample 1 DNA (2) 2nd blood sample 1 DNA (3) 1st blood sample 2 DNA (4) 2nd blood sample 2 DNA (5) 2nd blood sample 3 DNA (6) 2nd blood sample 3 DNA