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Comparison of Four Different DNA Extraction Methods and their Compatibility with Present-day STR Based Capillary Electrophoresis Typing

Neha Gupta[1], Rajesh Kumar[2] and Kunwar Veer Vikram Srivastav[3]*

[1]*Assistant Professor, Guru Ghasidas University, Bilaspur, Chhattisgarh, India*
[2]*Assistant Director, DNA Division, State Forensic Science Laboratory, Rajasthan, Jaipur, India*
[3]*Forensic Scientific Assistant, DNA Division, State Forensic Science Laboratory, Rajasthan, Jaipur, India*

Abstract

Currently, Short Tandem Repeats (STRs) based forensic DNA typing technology is being internationally used in solving a diverse range of forensic cases, and presently, forensic DNA tests employ Polymerase Chain Reaction (PCR) and Capillary Electrophoresis (CE) based fragment analysis methods to detect length variation in STRs. The quality of STR-based DNA profiles from challenging samples depends on the yield and quality of DNA. The yield of DNA predominantly depends upon the method used for DNA extraction. DNA extraction is the first and perhaps most important step in any Forensic DNA analysis. Any specific method can never be thought of to be convenient for all types of samples. Still, Phenol Chloroform Isoamyl Alcohol (PCIA) method which is called the organic extraction method has been proven to be useful for a wide variety of forensic samples ranging from the simplest saliva or blood to complex bone and teeth samples. In the present study, we compared the performance and DNA yield of four different DNA extraction methods- EZ1Advanced XL, AutoMate Express TM, PCIA, and chelax method on broad range of samples commonly encountered in forensic casework and their compatibility with present-day STR based capillary electrophoresis typing. The mean value of DNA yield was found 114.21 ng/µl, 49.028 ng/µl, 35.485 ng/µl, 4.694 ng/µl by PCIA, AutoMate Express TM, EZ1Advanced XL, and Chelex DNA extraction methods, respectively. Conclusively, the highest yield was observed from all the tested samples from the PCIA method.

Keywords: PCIA • AutoMate express TM • EZ1 advanced XL • Chelex DNA extraction methods • Capillary electrophoresis

Introduction

Short Tandem Repeats (STRs) based forensic DNA typing technology is being internationally used in solving a diverse range of forensic cases and presently, forensic DNA tests employ Polymerase Chain Reaction (PCR) and Capillary Electrophoresis (CE) based fragment analysis methods to detect length variation in STRs. The quality of STRs DNA profiles from challenging samples depends upon the yield and quality of DNA and yield of DNA is basically dependent upon the method used for DNA extraction [1,2]. DNA extraction is the first and most important step in any Forensic DNA analysis. Human DNA can be extracted from all the nucleated cells such as hair, tissue, blood etc. The DNA extraction of forensic sample is a critical step in the analysis process and plays a significant role in the success of next applications. The wide range of samples processed in forensic casework, which contains varying amounts of DNA, require a robust DNA extraction method [3-5]. DNA was first extracted by Friedrich Miescher who named it "nuclein" in 1869 and currently it is a routine procedure in forensic casework as well as molecular biology [4]. There are various methods of extraction of DNA. The Phenol-chloroform Method (PCIA) of DNA extraction is a well-established procedure of forensic DNA fingerprinting laboratories. It is known to be laborious compared with alternative approaches like magnetic beads based DNA extraction and it involves toxic reagents, such as phenol, which requires special safety precautions in the laboratory (laminar flow fume hood). But, phenol–chloroform extraction method is still the gold standard choice for samples containing very little amounts of DNA or samples suspected to contain Polymerase Chain Reaction (PCR)-inhibiting substances. Although this method involves intensive manual interaction-the sample is handled through at least three generations of reaction tubes—the DNA recovery of the phenol-chloroform extraction is known to be relatively high [6]. Chelex method of extraction was introduced in 1991 to the forensic DNA community. Chelex is an ion-exchange resin that is added as a suspension to the samples and it is composed of styrene divinylbenzene copolymers containing paired iminodiacetate ions that act as chelating groups in binding polyvalent metal ions such as magnesium. By removing the magnesium from the reaction, DNA destroying enzymes known as nucleases are inactivated and the DNA molecules are thus protected. Chelex extraction procedures for recovering DNA from bloodstains or semen containing stains are not effective for RFLP typing because Chelex denatures double-stranded DNA and yields single-stranded DNA from the extraction process. Thus, it can only be followed by PCR-based analysis [7]. Now-a-days, DNA extraction instruments are routinely used for obtaining DNA from various forensic samples [8]. Magnetic beads are more suitable for automation than conventional column methods; that is why magnetic beads are used in small and large scale DNA extraction instruments. The Bio Robot EZ1 (QIAgen, Hilden, Germany) is widely used in forensic DNA laboratories because of its ease of use, less time taking and flexibility [8-10]. Recently, the AutoMate Express TM (Applied Biosystems, Foster City, CA) was developed, it has the same ease of operation as the EZ1 series but it takes more time than EZ1. The magnetic particles are much smaller in comparison with other commonly used magnetic particles, which results in a larger surface area with a higher DNA binding capacity [9]. In our previous study (Table 1), we compared PCIA and chelex method of extraction for their efficiency in extracting DNA from bloodstains on various surfaces [11].

Present study involves comparative analysis of performance and DNA

***Address for Correspondence:** Kunwar Veer Vikram Srivastav, Forensic Scientific Assistant, DNA Division, State Forensic Science Laboratory, Rajasthan, Jaipur, India, Tel: +919792067065, E-mail: kunwarsrivastav678@gmail.com*

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Table 1. Average yield of DNA (ng/µl) from various samples using PCIA, automate express TM, and EZ1 advanced XL and Chelex method.

S. No.	Sample	Amount	Phenol-Chloroform Extraction	Automate Express TM	EZ1 Advanced XL	Chelex Extraction
1	Semen (Liquid)	50 µl	536.7909 ng/µl	184.5211 ng/µl	101.9156 ng/µl	10.85961 ng/µl
2	Nail clipping	15 mg	88.58139 ng/µ	58.35163 ng/µl	30.42272 ng/µl	0.795589 ng/µl
3	Chest hair	1 (3.5 cm)	3.956289 ng/µ	1.324459 ng/µl	1.039988 ng/µl	0.346774 ng/µl
4	Blood stain on T-shirt (Terrycot)	1 × 1 cm	128.9829 ng/µ	87.30354 ng/µl	59.65596 ng/µl	10.16022 ng/µl
5	Blood stain on gauge	1 × 1 cm	250.4594 ng/µ	87.08136 ng/µl	89.38024 ng/µl	1.902488 ng/µl
6	Public Hair	1 (3 cm)	12.17523 ng/µ	5.115998 ng/µl	2.206983 ng/µl	0.373759 ng/µl
7	Blood stain on Baniyan (Cotton)	1 × 1 cm^2	181.4622 ng/µ	101.5098 ng/µl	73.34391 ng/µl	14.64154 ng/µl
8	Semen stain (Cotton)	1 × 1 cm^2	318.9606 ng/µ	89.57643 ng/µl	75.1764 ng/µl	6.954396 ng/µl
9	Blood stain on inner (Woollen)	1 × 1 cm^2	97.20501 ng/µ	48.35943 ng/µl	62.31758 ng/µl	5.615016 ng/µl
10	Hair (From canal)	6 cm	1.539087 ng/µ	0.661917 ng/µl	0.5917628 ng/µl	0.389568 ng/µl
11	Half singed hair	6 cm	3.936004 ng/µ	1.414296 ng/µl	2.485101 ng/µl	0.282296 ng/µl
12	Blood (Liquid)	40 µl	199.3071 ng/µ	118.0767 ng/µl	68.19788 ng/µl	22.7131 ng/µl
13	Saliva stain (Paper)	1 × 1 cm^2	2.859168 ng/µ	0.602129 ng/µl	0.527838 ng/µl	0.016886 ng/µl
14	Cigarette butt (with filter)	0.5 mm^2	0.121715 ng/µ	0.008866 ng/µl	0.010279 ng/µl	0.001343 ng/µl
15	Cigarette butt (without filter)	0.5 mm^2	0.200838 ng/µ	0.543836 ng/µl	0.099436 ng/µl	0.00287 ng/µl
16	Aspermic semen	50 µl	0.825395 ng/µ	0.008429 ng/µl	0.398694 ng/µl	0.049771 ng/µl

yield of four different DNA extraction methods- EZ1 advanced XL, AutoMate Express TM, PCIA and Chelex method on broad range of samples commonly encountered in forensic caseworks.

Materials and Methods

In the present study, triplicate of sixteen variety of routine casework and simulated samples including Semen (liquid), Nail Clipping, Chest Hair, Blood stain on T-Shirt (Terrycot), Blood stain on Gauge, Pubic Hair, Blood stain on Baniyan (cotton) Semen Stain on cloth, Blood Stain on Inner (wool) Hair (from canal), Half Singed Hair, Blood (liquid), Saliva stain (paper), Cigarette butt (with filter), Cigarette butt (without filter) and Aspermic semen were subjected DNA extraction in equal amount using PCIA, Automate express TM, EZ1 advanced XL and Chelex DNA extraction methods as per standard recommendations (Figure1) [12-15] .

Quantitative PCR

To assess DNA yields, we used Quantifiler® DNA Quantification Kit (Thermo Fisher Scientific, USA) using Real-Time PCR 7000 system (Thermo Fisher Scientific, USA).

Results and Discussion

The average value of DNA yield (ng/µl) from Semen(liquid), Nail Clipping, Chest Hair, Blood stain on T-Shirt (Terrycot), Blood stain on Gauge, Pubic Hair, Blood stain on Baniyan (cotton) Semen Stain (cotton), Blood Stain on Inner (woolen), Hair(from canal), Half Singed Hair, Blood (liquid), Saliva stain (paper), Cigarette butt (with filter), Cigarette butt (without filter) and Aspermic semen were 536.7909, 88.58139, 3.956289, 128.9829, 250.4594, 12.17523, 181.4622, 318.9606, 97.20501, 1.539087, 3.936004, 199.3071, 2.859168, 0.121715, 0.200838 and 0.825395 (ng/ µl) respectively from the Phenol-Chloroform Isoamyl Alcohol (PCIA) extraction method. The average value of DNA yield (ng/µl) from Semen(liquid), Nail Clipping, Chest Hair, Blood stain on T-Shirt(Terrycot), Blood stain on Gauge, Pubic Hair, Blood stain on Baniyan (cotton) Semen Stain (cotton), Blood Stain on Inner (woolen), Hair (from canal), Half Singed Hair, Blood(liquid), Saliva stain (paper), Cigarette butt (with filter), Cigarette butt(without filter) and Aspermic semen were 184.5211, 58.35163, 1.324459, 87.30354, 87.08136, 5.115998, 101.5098, 89.57643, 48.35943, 0.661917, 1.414296, 118.0767, 0.602129, 0.008866, 0.543836 and 0.008429 (ng/µl) respectively from the Automate Express. The average value of DNA yield (ng/µl) from Semen(liquid), Nail Clipping, Chest Hair, Blood stain on T-Shirt (Terrycot), Blood stain on Gauge, Pubic Hair, Blood stain on Baniyan (cotton) Semen Stain (cotton), Blood Stain on Inner (woolen), Hair(from canal), Half Singed Hair, Blood(liquid), Saliva stain (paper), Cigarette butt (with filter), Cigarette butt(without filter) and Aspermic semen were 101.9156, 30.42272, 1.039988, 59.65596, 89.38024, 2.206983, 73.34391, 75.17647, 62.31758, 0.591762, 2.485101, 68.19788, 0.527838, 0.010279, 0.099436 and 0.398694(ng/µl) respectively from the EZ1. The average value of DNA yield (ng/µl) from Semen (liquid), Nail Clipping, Chest Hair, Blood stain on T-Shirt (Terrycot), Blood stain on Gauge, Pubic Hair, Blood stain on Baniyan (cotton) Semen Stain (cotton), Blood Stain on Inner (woolen), Hair (from canal), Half Singed Hair, Blood (liquid), Saliva stain (paper), Cigarette butt (with filter), Cigarette butt (without filter) and Aspermic semen were 10.85961, 0.795589, 0.346774, 10.16022, 1.902488, 0.373759, 14.64154, 6.954396, 5.615016, 0.389568, 0.282296, 22.7131, 0.016886, 0.001343, 0.00287 and 0.049771(ng/µl) respectively from the Chelex method.

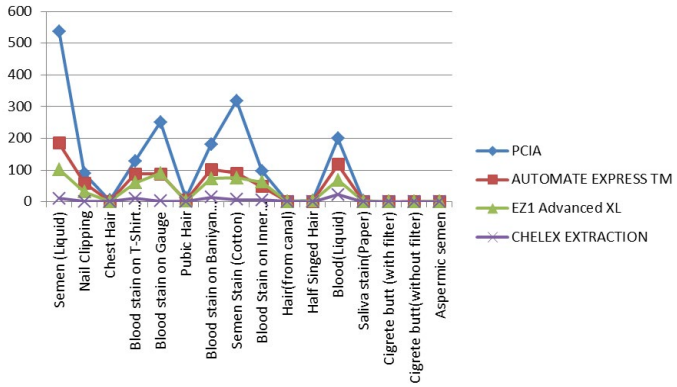

Figure 1. Relative values of the final comparison of average yield of DNA (ng/µl) from various samples using PCIA, automate express TM, and EZ1 advanced XL and Chelex method of DNA isolation.

The highest average yield of DNA (536.7909 ng/µl) was obtained from liquid semen whereas the lowest average yield of DNA (0.121715 ng/µl) was obtained from cigarette butt (with filter) with the use of the PCIA method. From the Automate Express, the highest average yield of DNA (184.5211 ng/µl) was obtained from liquid blood whereas the lowest average yield of DNA (0.008429 ng/µl) was obtained from the cigarette butt (with filter). From the EZ1, the highest average yield of DNA (101.9156 ng/µl) was obtained from liquid blood whereas the lowest average yield of DNA (0.010279 ng/µl) was obtained from the cigarette butt (with filter). In the Chelex method, the highest average yield

of DNA (22.7131 ng/μl) was obtained from liquid blood whereas the lowest average yield of DNA (0.001343 ng/μl) was obtained from the cigarette butt (with filter). Comparatively higher DNA yield was observed in DNA extraction using PCIA method for all the samples.

Conclusion

The yield of DNA from Semen(liquid), Nail Clipping, Chest Hair, Blood stain on T-Shirt(Terrycot), Blood stain on Gauge, Pubic Hair, Blood stain on Baniyan (cotton) Semen Stain (cotton), Blood Stain on Inner (woolen), Hair(from canal), Half Singed Hair, Blood (liquid), Saliva stain (paper), Cigarette butt (with filter), Cigarette butt(without filter) and Aspermic semen encountered in various crimes like murder, rape etc. was observed highest in Phenol Chloroform Isoamyl Alcohol (PCIA) method than the Automate Express, EZ1 and Chelex method. The observations of the study strongly suggest that for the forensic samples, where chance of getting DNA is low, the Phenol Chloroform Isoamyl Alcohol (PCIA) method should be preferred but if the quality and quantity sample is good then we can use Automate express and EZ1 in the place of PCIA.

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