International Congress Series 1288 (2006) 700–703

www.ics-elsevier.com

Sperm DNA extraction from mixed stains using the Differex™ System

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Abstract. We compared the extraction efficiency of the Differex™ System and the two-step method. Sperm DNA extracted from mixed stains were amplified with the AmpFlSTR® Profiler PCR Amplification Kit. Electrophoresis was performed with an ABI 310 Genetic Analyzer, and alleles were determined with GenoTyper 3.7 software. The results indicate that the Differex™ System offers efficiency equal to the two-step method for extracting sperm DNA from mixed stains.

Keywords: Sexual crime; Mixed stain; STR; Sperm DNA; Differex system

1. Introduction

DNA typing is a powerful and important tool in criminal investigations, particularly sexual crimes. Although virtually all crime specimens are blood or blood stains, specimens from rape cases generally consist of mixed stains, such as sperm-oral cells or sperm-vaginal cells. Multiplex PCR kits for Y-STR introduced in recent years enable rapid typing of numerous sperm loci. However, for autosomal DNA typing of sperm from mixed stains, we must extract just the sperm DNA from mixed stains by the two-step method (or the two-step differential extraction procedure) [1]. The two-step method is time consuming, requiring at least 1 or 2 days.

A new kit called the Differex™ System has recently been introduced by Promega Co. The Differex™ System combines phase separation and differential centrifugation to separate sperm and epithelial DNA. The system offers the potential for rapid extraction of

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0531-5131/
doi:10.1016/j.ics.2005.12.059

sperm DNA from mixed stains (in approximately 2–3 h)—significantly faster than earlier methods.

In this experiment, mixed stains consisting of female epithelial cells and sperm at various concentrations were deposited on cotton to compare the extraction efficiency of this system and the two-step method.

2. Materials and methods

2.1. Formulating the mixed stains

HeLa cultured cells were used as female epithelial cells. Semen was obtained from a healthy male, and the sperm was prepared by washing the semen with phosphate-buffered saline (−) following semen liquefaction. HeLa cells and sperm were counted with a counting chamber. The mixed stains were deposited on cotton by mixing the female epithelial cells and the sperm at various concentrations. The mixed ratios of female epithelial cells to sperm were 500:0, 500:50, 500:500, 500:5,000, 500:50,000, 500:500,000, 0:500, respectively.

2.2. DNA extraction

Sperm DNA was extracted by the four following methods: (a) Differex™ System+-DNA IQ™ System, (b) Differex™ System+QIAamp® DNA Micro Kit, (c) Differex™ System+phenol/chloroform extraction, (d) Two-step method. Epithelial DNA and sperm DNA were separated from mixed stains using the Differex™ System, and DNA purification was performed with the DNA IQ™ System. Both systems were used according to the instructions provided by Promega Co., the manufacturer [2,3]. DNA purification was performed using the QIAamp® DNA Micro Kit according to the instructions provided by QIAGEN Inc., the manufacturer [4]. DNA extracted by each of the preceding four methods was dissolved in 25 μl each of TE^{-4}.

2.3. PCR amplification

PCR reactions were performed according to manufacturer's recommendations using the AmpFlSTR® Profiler Amplification kit (AppliedBiosystems, Foster City, CA, USA) [5]. To compare the extraction efficiency of the four methods, PCR amplification was performed using 1 μl of extracted DNA, given the difficulty of estimating DNA concentrations due to the small quantity of original extracted DNA. Signal detection was performed using the ABI Prism® 310 Genetic Analyzer. Alleles were determined with GenoTyper 3.7 software.

3. Results

Allele typing was successful for sperm counts of 50,000 and 500,000 by all DNA extraction methods. For the case of 50,000 sperm, the highest peak remained below 2000 RFU (relative fluorescent units) regardless of DNA extraction methods. Comparing peak heights for the four methods, we observed the highest peaks with the Differex™ System+QIAamp® DNA Micro Kit combination (Fig. 1). The lowest peaks were observed with the Differex™ System+phenol/chloroform extraction combination. The

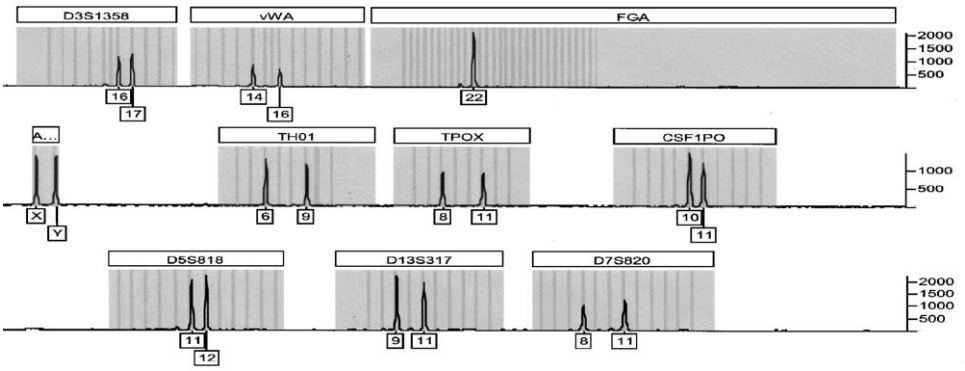

Fig. 1. Electropherogram of amplified sperm DNA from mixed stain. PCR amplification was performed using 1 μl of DNA extracted with the Differex™ System+QIAamp® DNA Micro Kit as template.

case of 500,000 sperm resulted in a peak height exceeding 6000 RFU. No signal peaks were observed with sperm counts lower than 5000. Allele typing of female epithelial cells was successful with all types of mixed stains and all four extraction methods. The Differex™ System significantly reduced the time required for sperm DNA extraction from mixed stains: from 1–2 days to a mere 2–3 h. The QIAamp® DNA Micro Kit provided a DNA yield superior to the DNA IQ™ System, although it required slightly more time.

4. Discussion

To compare extraction efficiency, we extracted DNA from mixed stains by four methods. In this study, allele typing was completely successful for sperm counts exceeding 50,000 with all four DNA extraction methods. No signal peaks were observed with sperm counts below 5000. We regarded 50,000 to constitute an adequate sperm count, since the ejaculate volume for healthy males is generally 2 to 6 ml (average: 3 ml), and sperm concentrations are $50–150 \times 10^6$ sperm/ml (average: 80×10^6 sperm/ml) [6].

The signal peak heights suggest that extraction efficiency is generally equivalent for the three new methods with the exception of the Differex™ System+phenol/chloroform combination, which produced the lowest signal peak height. The Differex™ System separation solution must be removed from the sperm pellet before the addition of the digestion solution containing Proteinase K and DTT, since the separation solution is not water-soluble. Apparently, some sperm is lost when the solution is removed. When the low peak height was detected, the peak could be heightened by increasing the template DNA for PCR amplification.

Our results suggest that the Differex™ System, offering dramatically faster DNA extraction and easy separation of female epithelial DNA and sperm DNA, represents a potentially useful tool for procedures requiring the processing of sexual assault samples.

References

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