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Research article

Evaluation of the Differex™ System

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Abstract

Differential extraction is an efficient method to separate sperm cells from epithelial cells. A manual Chelex®-100 based method is used at the Swedish National Laboratory of Forensic Science, SKL. The Differex™ System (Promega) uses a Proteinase K digestion of epithelial cells followed by centrifugation and phase separation. The sperm- and epithelial fractions are further purified with DNA IQ™ System (Promega) or with phenol/chloroform. The Differex™ System in combination with DNA IQ™ System were evaluated and compared to the Chelex®-100 method used routinely. After modifications, the Differex™ System gave comparable results to the Chelex®-100 method. The modifications included additional Proteinase K and DTT, longer incubation time and additional steps when removing the solid support from the Digestion Solution. In the Chelex®-100 based method microscopic examination is done on the sperm pellet in a total volume of 50 μl. It was not possible to do a microscopic examination in less than 100 μl using the Differex™ System. Additionally the sperms were in clusters of epithelial cell debris. Microscopic examination is an important part of the differential extraction at SKL. Therefore, the Differex™ System will not be implemented at our laboratory.

Keywords: Extraction; Differential lysis; Differex™ System; Sperm

1. Introduction

Samples from sexual assault cases often include a mixture of epithelial cells from the victim and sperm cells from the assailant. Differential lysis is an efficient method to separate sperm cells from epithelial cells. A manual Chelex®-100 based method is used at the Swedish National Laboratory of Forensic Science, SKL. The Differex™ System (Promega) uses a Proteinase K digestion of epithelial cells followed by centrifugation and phase separation. The sperms form a pellet at the bottom of the tube, in the non-aqueous separation solution. The epithelial DNA remains in the above yellow aqueous digestion buffer. The sperm- and epithelial fractions are further purified with DNA IQ™ System (Promega) or with phenol/chloroform. The total time from a sample to a purified extract, using the Differex™ System and DNA IQ™ System, is 2 h. In this project the Differex™ System in combination with DNA IQ™ System were evaluated and compared to the Chelex®-100 method used routinely.

2. Materials and methods

Simulated crime scene samples were prepared by transferring sperms (approximately 55,000 sperms) and female epithelial cells to a cotton swab in a 1.5 ml tube. Samples were extracted with the Chelex®-100 based method [1], which includes Proteinase K (500 μg/ml) digestion of epithelial cells. The Differex™ System was tested during four phases, one standard and three modified methods. All fractions were purified using DNA IQ™ System.

In Phase I, samples were run according to the Promega instructions (Proteinase K: 270 μg/ml). Phase II modifications; after lysis of the epithelial cells, the tube was vortexed and the solid support was removed after intensive whirling with a small stick. Proteinase K: 500 μg/ml. Phase III modifications; same as in phase II plus longer incubation time for the lysis of the sperm cells (from 5 to 30 min). Increased DTT concentration from 0.0067 to 0.04 M in the sample. Phase IV modifications; same as in phase III plus no removal of the separation solution. In the instructions a part of the separation solution is removed during the extraction. The separation solution can, according to Promega, include some sperms.

Microscopic preparation was performed according to the Promega instructions [2] and stained using the ‘‘Christmas

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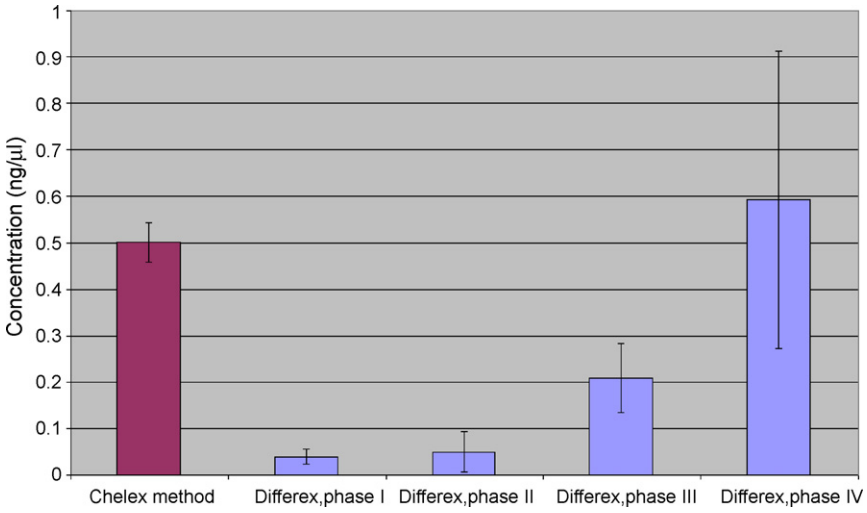

Fig. 1. The mean concentration of five sperm fractions for the Chelex®-100 method and phase I, and four sperm fractions for phases II–IV together with the standard deviation.

Tree'' technique [3]. The DNA analysis was performed using 7300 Real Time PCR System with Quantifier™ Human DNA Quantification Kit, GeneAmp® PCR System 9700 with AmpFISTR® SGM Plus® PCR Amplification Kit, Applied Biosystems 3130xl Genetic Analyzer and GeneMapper™ *ID* v3.1 (Applied Biosystems).

3. Results and discussion

The sperm fractions from phase I all gave mixed profiles so in phase II the focus was to optimize the epithelial cell lysis. The sperm fractions in phase II gave male profiles with only a minor contribution of female DNA, which is also seen in the Chelex®-100 method. However, the concentration was still quite low compared to the Chelex®-100 method (Fig. 1). Modifications in phases III and IV were made to optimize the sperm lysis. The epithelial fractions gave high concentrations and female profiles.

In the Chelex®-100 based method, microscopic examination is done on 3 μl of the sperm pellet from a total volume of 50 μl. The Differex™ System requires a total volume of at least 100 μl. Since the verification of sperms forms an evidence on its own, it is important to perform the microscopic examination in a small total volume. The separation solution formed droplets that were very difficult to dry and the slide had to be heated for a long time before staining. The droplet formation also made it impossible to obtain an even smear for staining. Additionally the sperm heads were found in clusters with epithelial cell debris, making the examination procedure difficult.

4. Conclusion

After modifications, the Differex™ System gave comparable results to the Chelex®-100 method used routinely in the laboratory. Microscopic examination is an important part of the differential extraction at SKL. Since the microscopic preparation did not give satisfactory results the Differex™ System will not be implemented at SKL.

Conflict of interest

None.

References

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