Differential Extraction of Mixtures in Sexual Assault Casework Using Pressure Cycling Technology (PCT)

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ABSTRACT

- Conventional differential extraction methods used for the separation of DNA from sperm cells and epithelial cells in sexual assault casework rely on a complicated procedure of selective digestion and separation of epithelial cell fractions followed by sperm cell lysis to generate the male genetic profile.

Organic Differential Extraction

- Incomplete digestion of Epithelial cells
- Initial digestion of Sperm cells
- Laborious

- The objective of this study is to develop a selective pressure-based DNA extraction procedure that is designed to burst open and extract DNA from male sperm cells while leaving the excess female cells in rape kits or other mixed forensic stains unaffected.

Pressure-based extraction

- Shorter analysis time
- Automation
- User-controlled variables

MATERIALS AND METHODS

SAMPLES
Sperm cells and Epithelial cells suspended in 1X PBS buffer (pH 7.4)

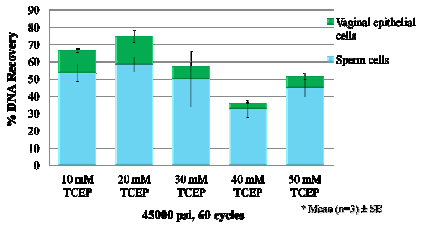
→ **CELL LYSIS**
Pressure cycling using Barocycler® NEP 2320

→ **PURIFICATION**
Phenol-Chloroform-Isoamyl alcohol extraction

→ **QUANTITATION**
Real-time PCR analysis using Plexor® HY system (Promega Corporation)

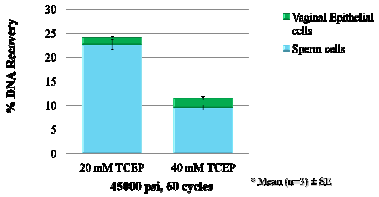
RESULTS AND DISCUSSION

Figure 1. Effect of PCT on individual cell types (TCEP)



TCEP produced an increase in selectivity between sperm cell and epithelial cell lysis

Figure 2. Effect of PCT on solubilized mixtures (TCEP)



The selectivity between sperm cell and epithelial cell lysis is reproduced with pressure cycling extraction of TCEP-treated mixtures

Figure 3. Effect of PCT on solubilized mixtures in the presence of DTT and increased number of cycles

- Addition of DTT to TCEP improved selectivity of extraction.
- Increasing the number of pressure cycles to above 60 did not improve recovery

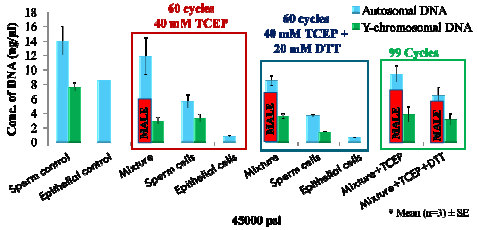
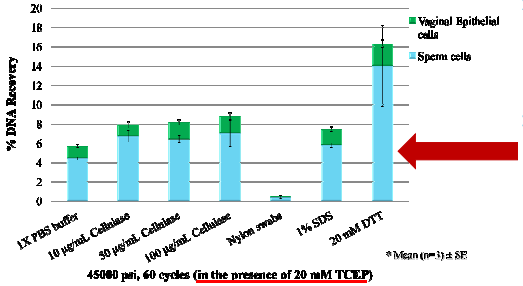


Figure 4. Effect of PCT on DNA extraction from swab (mixture of sperm and vaginal epithelial cells)



- Incubation of swabs with Cellulase (*A.niger*) or 1% SDS (42°C, 2 hours) caused a slight increase in yields compared to TCEP treatment alone
- Incubation with 20 mM DTT (42°C, 2 hours) followed by TCEP treatment caused significant improvement in sperm DNA recovery from swabs while maintaining selectivity

CONCLUSIONS

- Depending on buffer component epithelial or sperm cells can be selectively lysed with PCT treatment
- TCEP produces improved selectivity of sperm extraction.
- Combining TCEP and DTT further improves sperm DNA yield in case of mixed samples
- Further studies need to be done to optimize yield from dried stains by examining temperature and buffer concentration
- Another important aspect of future research is solubilizing and examining dried stains – measuring yield for removal of cells from swabs and fabric samples

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