UNIVERSITY of NORTH TEXAS
HEALTH SCIENCE CENTER
AT FORT WORTH

PRESSURE CYCLING TECHNOLOGY (PCT): APPLICATIONS FOR FORENSIC DNA ANALYSIS

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Research Appreciation Day, 2010

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IDENTIFICATION
Laboratory of Forensic Anthropology • Laboratory for Molecular Identification

Current Forensic Need

- Challenged/Degraded Samples and Touch DNA
- Most studies focus on improving "downstream analysis"
- Low level DNA samples will not yield reproducible and reliable results
- "Garbage in, garbage out"

Purpose

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To increase DNA recovery from devices used for collecting crime scene biological evidence, such as cotton swabs

Samples containing various amounts of purified DNA or cells were placed onto swabs and processed with Pressure Cycling Technology (PCT).

Pressure Cycling Technology (PCT)

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- PCT is a novel technology that uses cycles of hydrostatic pressure between atmospheric and ultra-high levels (up to 35,000 psi)
- Allows for the precise control of biomolecular interactions

Pressure Cycling Technology (PCT)

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- PCT alters conformations and interactions of biomolecules
- Destabilizes secondary structures
- Does not denature or inhibit enzymes
- Does not appear to degrade DNA below levels practical for forensic analyses

The Barocycler NEP3229

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- Bench top instrument
- External chiller hook-up
- Uses a programmable logic controller
- Processes up to three samples simultaneously

The PULSE Tube

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Specially designed multi-functional tube:

- Single-use
- Versatile, works with:
  - Standard and custom reagents
  - Various sample types
  - Range of sample sizes
- Convenient
- Efficient
- Safe: closed tube, sample fully-contained

Study Design

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- Purified DNA or epithelial cells
- Samples processed with and without PCT
- Samples extracted using DNA IQ™ Casework Kit on the Maxwell® 16
- Samples quantified

Maxwell® 16 Instrument

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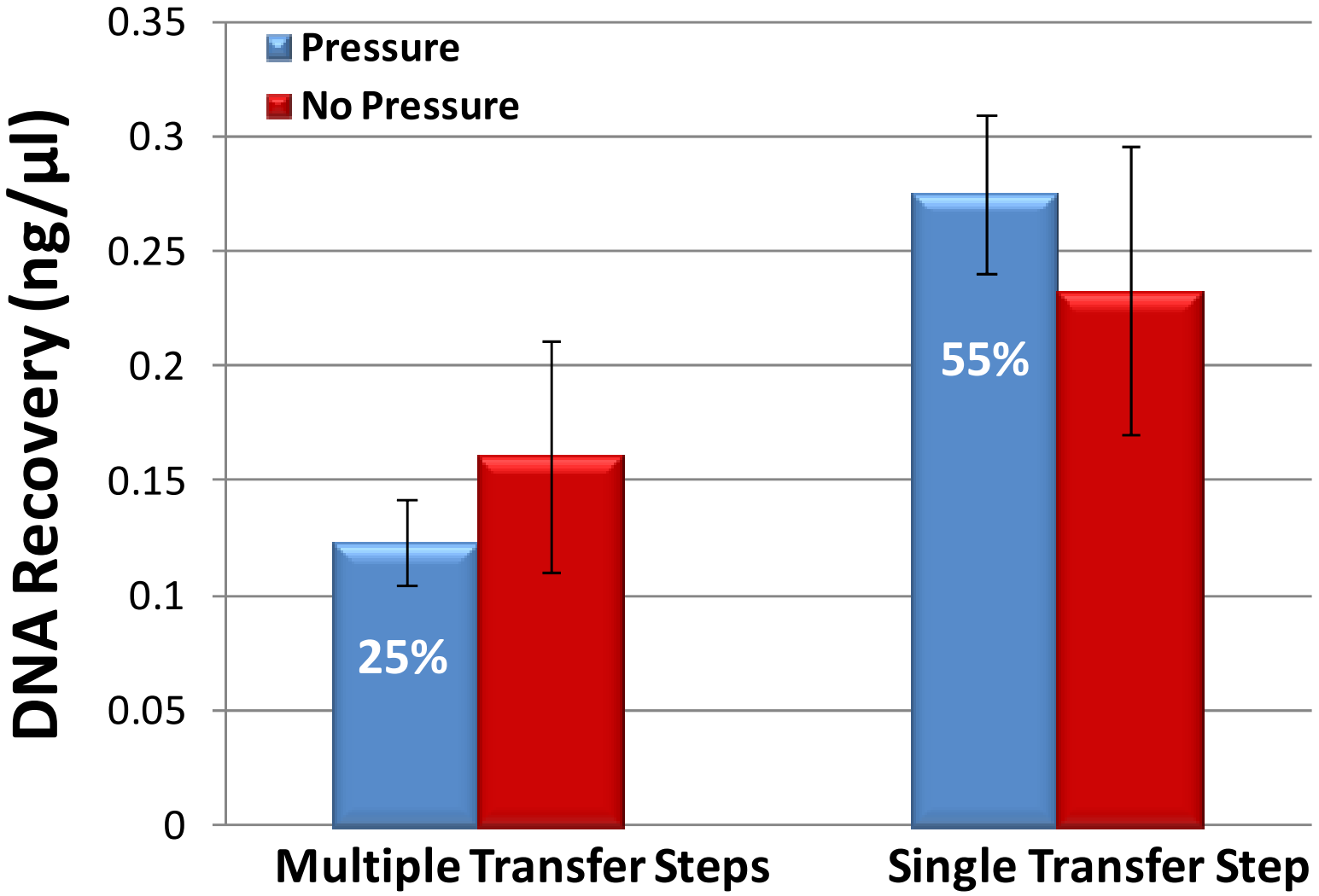
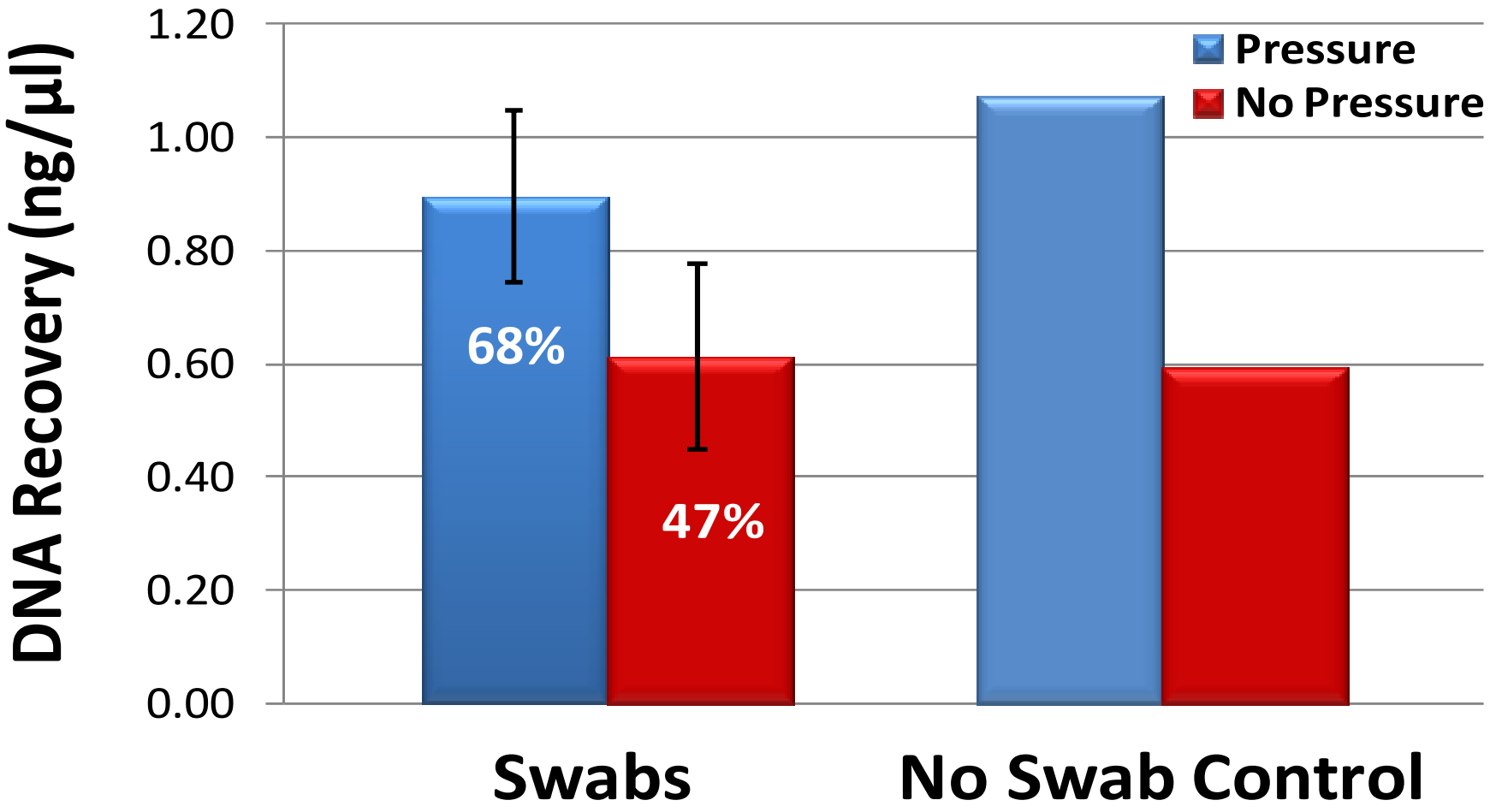
- Easy method for efficient, automated DNA purification
- Preprogrammed purification procedures
- Use with predispensed reagent cartridges
- Processes up to 16 samples in approximately 30 minutes

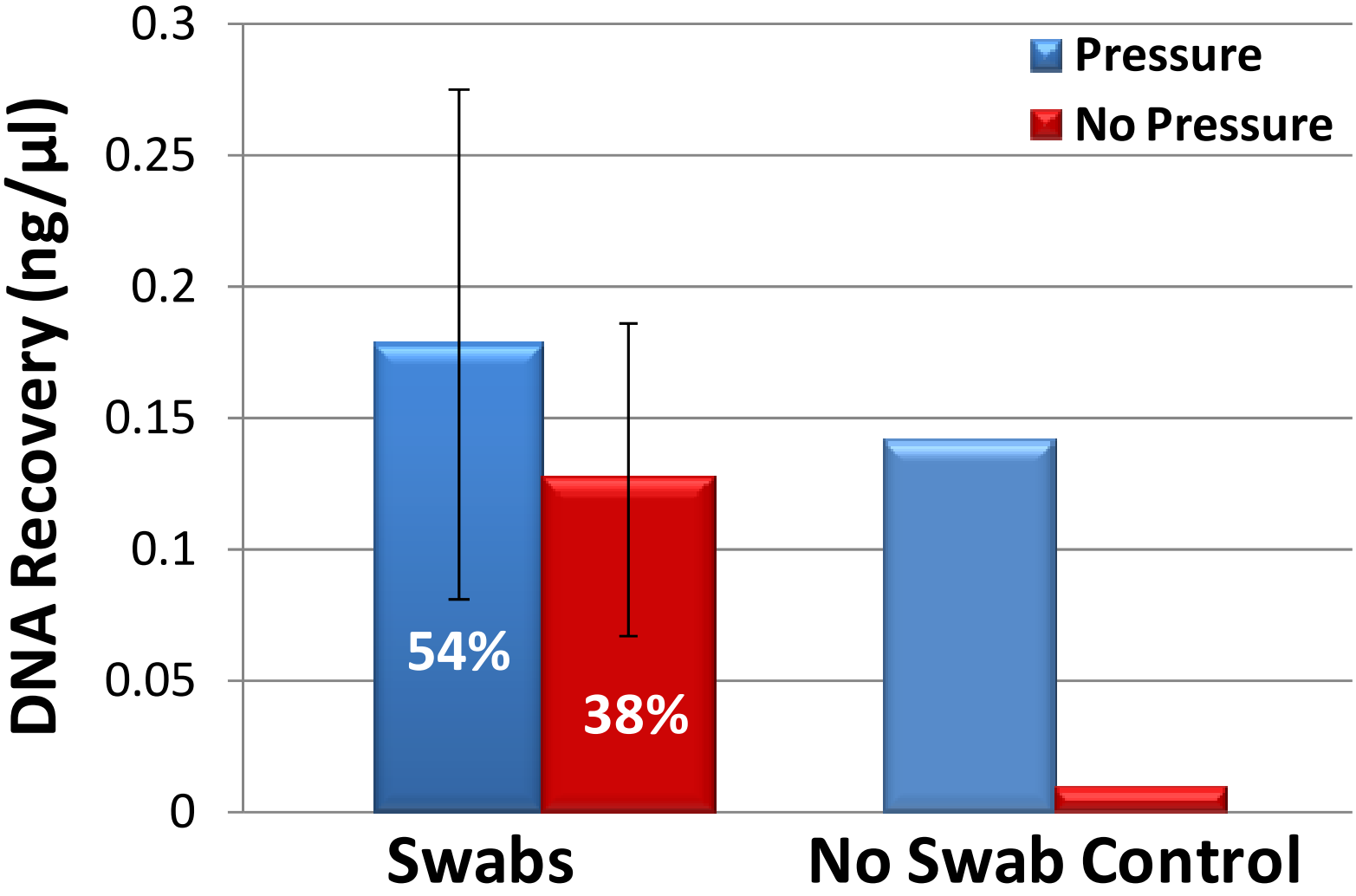
Tube Transfer
(Purified DNA Swabs)



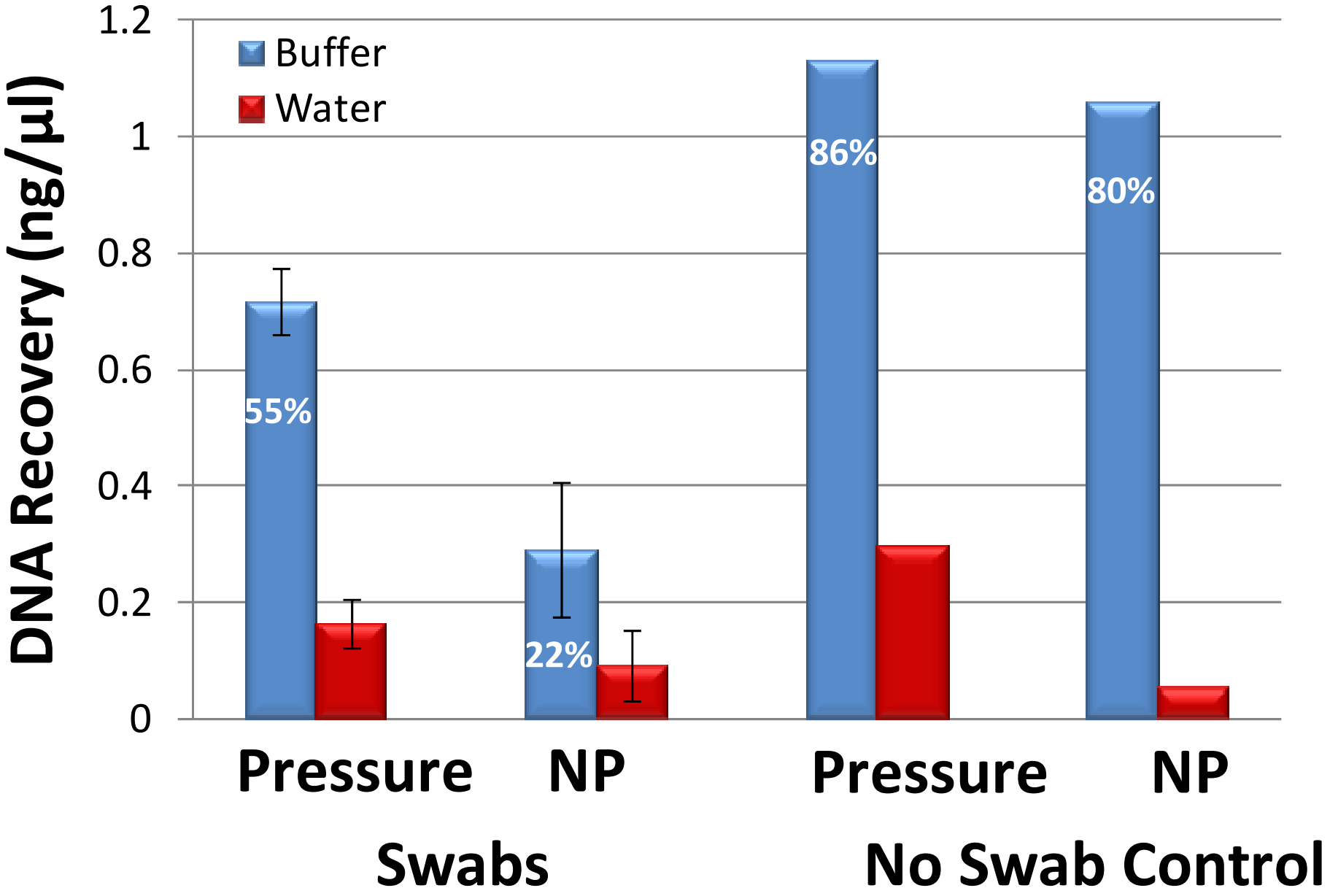
Pressure vs. No Pressure (200 cells/µl)



Pressure vs. No Pressure (50 cells/µl)



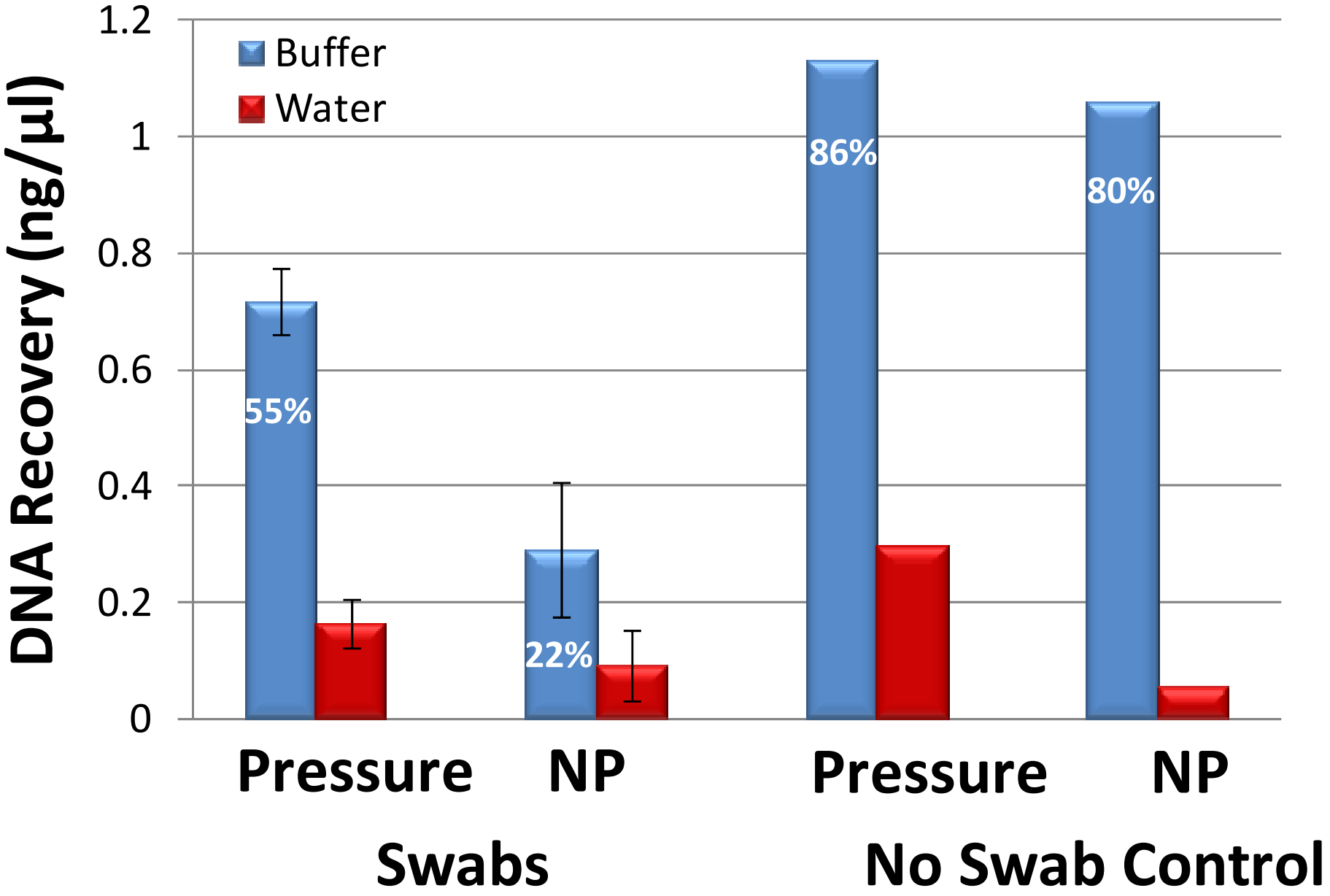
Buffer vs. Water



Additional Parameters Evaluated (Data Not Shown)

- Buffer Volumes (Incubation:Lysis Volumes – 1:1 vs. 1:2)
  - Results showed improved recovery with the 1:2 volume
- Tube siliconization
  - Results showed no difference in siliconized vs. non-siliconized PULSE tubes

Buffer vs. Water



Results Summary

- The data illustrate increased DNA yield in samples following PCT compared with those samples not exposed to pressure technology.
- These results indicate that PCT is a viable method to enhance DNA recovery from forensic samples.
- PCT can be used in conjunction with commercially available extraction reagents.

Conclusions

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- This research study demonstrates the capabilities and potential of PCT applications for enhancing robustness of forensic DNA analysis of biological evidentiary samples.
- The impact is that some samples that traditionally yield too little DNA for typing may now be suitable for routine analysis.
- More cases may be solved with this combined approach of PCT and DNA extraction.

Future Directions

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- White Blood Cells
- Collection Device
- Extraction Method
- Aqueous-Based Siliconizing Agent

Thank You!

Pressure BioSciences, Inc.

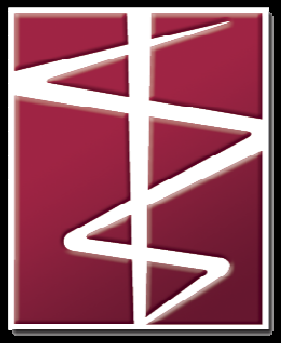
- Matt Potter
- Nate Lawrence

Promega

- Cami Green
- Kimberly Huston

Applied Biosystems

- Jaiprakash Shewale
- James Stray



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