

Evaluation of Direct PCR on Aged Touch DNA Cuttings & Swabs

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Introduction

- Direct polymerase chain reaction (PCR) is a method in which a swab or substrate punch is subjected to amplification without prior extraction or quantification.
- Direct PCR can reduce cost, labor, processing time, and DNA loss.
- Successful DNA typing can be affected by the collection method used, substrate the biological material is on, and storage time.
- This study examined whether direct PCR was effective on touch DNA evidence samples that were cut/swabbed and stored at room temperature for up to 6 months prior to processing.

Touch DNA Substrates



- 1" x 5/8" areas outlined on non-fabric items
- Three donors per non-fabric item; handled for 1 min
- One donor per fabric item; worn for ≥ 12 hours

Methods

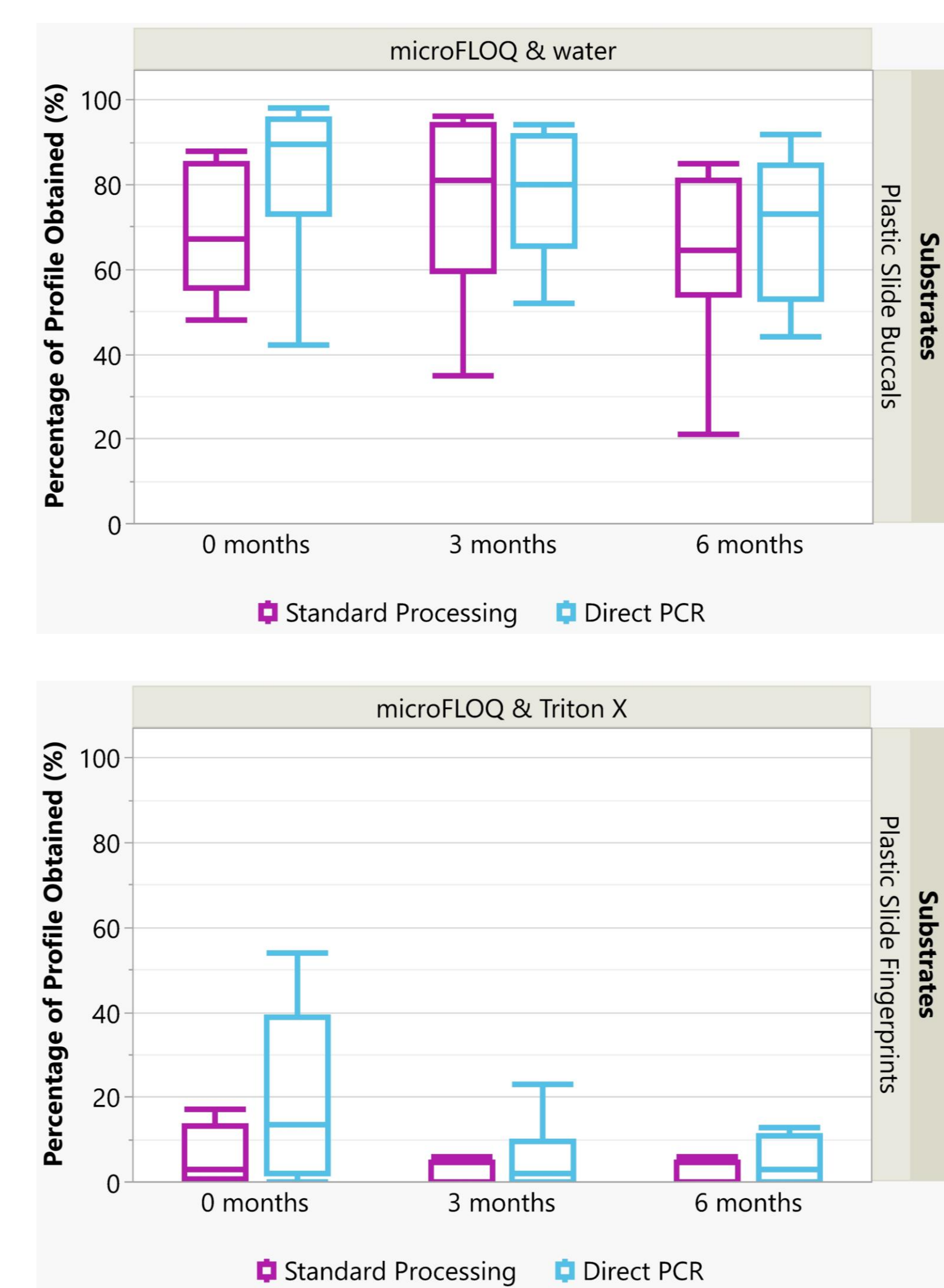
Collection Substrate	Diameter (mm ²)	Collection Method	Moistening Agent
Copan microFLOQ [®] swab	1.2–2.0	Swabbing	Sterile H ₂ O 0.1% Triton X
Fabric	2.0	Cutting	None - dry

- The collection method used for each substrate was determined based on prior testing.

- Two processing methods
 - Standard with DNA extraction and quantification
 - Direct PCR
- Three time points
 - 0 months
 - 3 months
 - 6 months
- Eight replicates per donor, collection method, processing method, & time point
 - n = 1,248

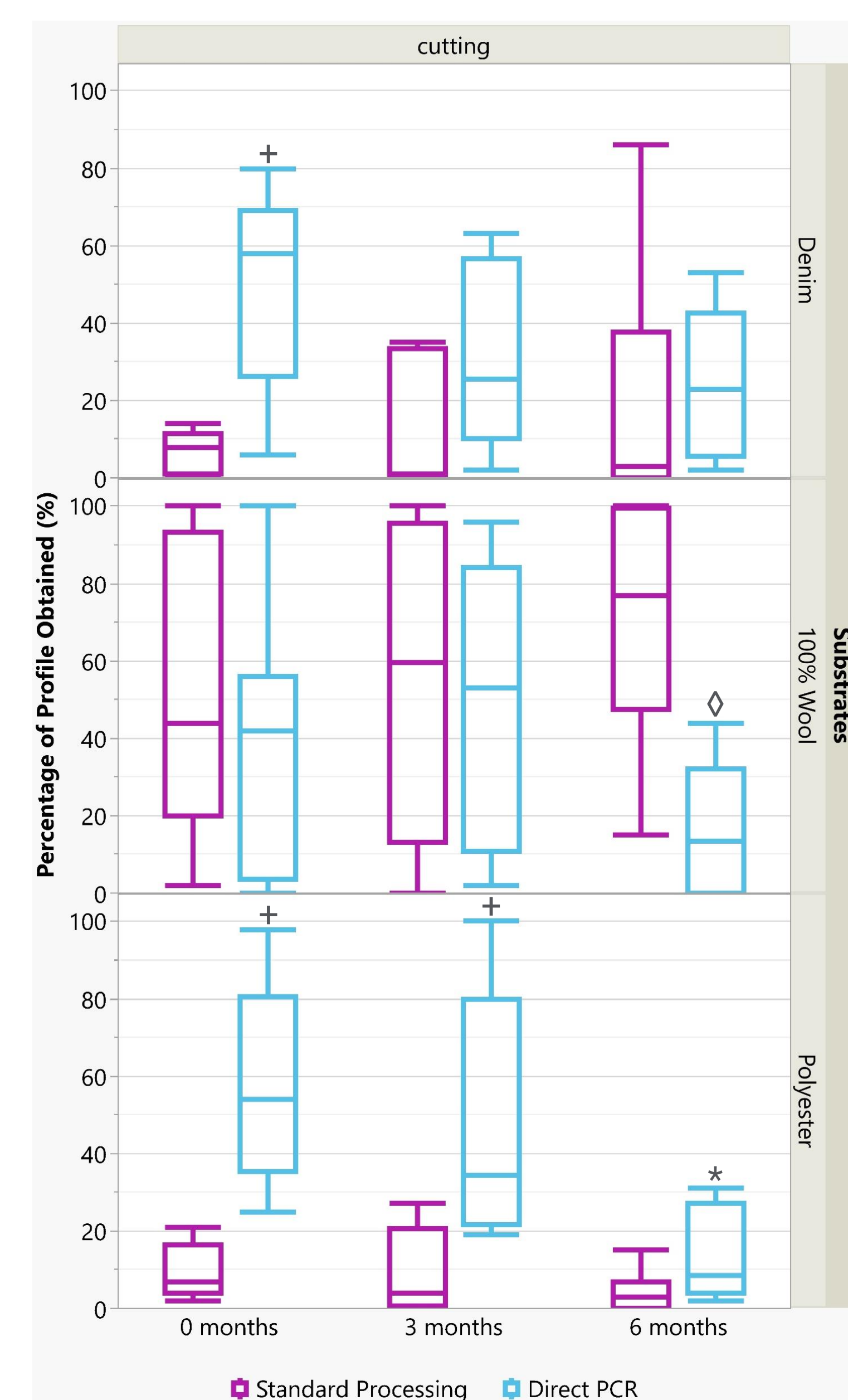
Process	Method
Extraction	Qiagen Investigator [®] STAR [™] Lyse&Prep Kit
Quantification	Quantifiler [®] Trio
Concentration	Microcon [®] DNA Fast Flow
Amplification	PowerPlex Fusion 6C [®] 25 µL, 29 cycles
CE	3500xL Genetic Analyzer
Data analysis	GeneMapper ID-X [®] v1.5 Analytical threshold 100 RFU Stochastic threshold 500 RFU
Evaluation metrics	Percentage of profile obtained (% profile) CODIS eligibility

Controls

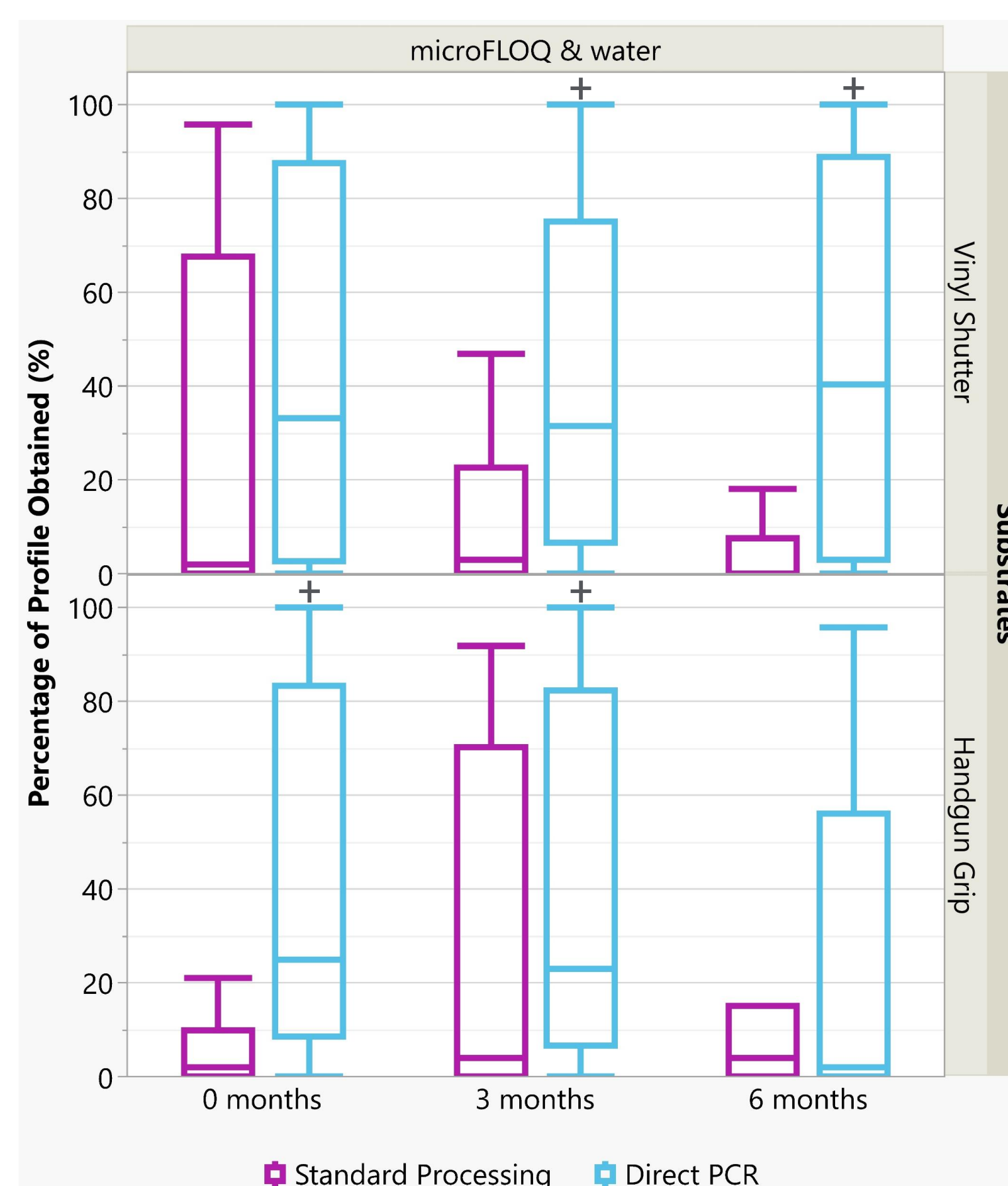
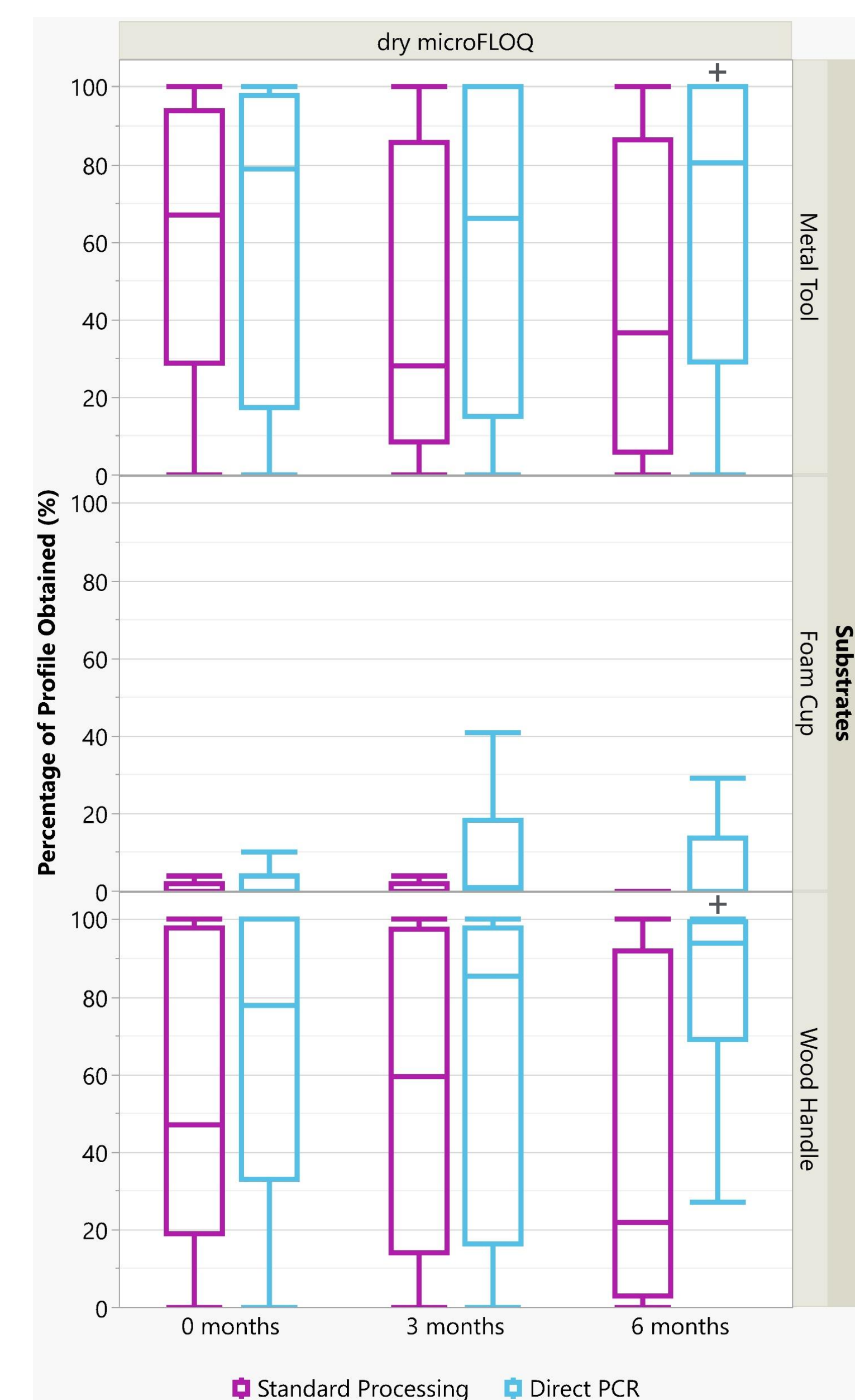


+ Direct PCR % profile significantly higher than standard ($p < 0.05$, Wilcoxon Test)
 ◇ Direct PCR % profile significantly lower than standard ($p < 0.05$, Wilcoxon Test)
 * Significantly lower % profile compared to 0 months ($p < 0.05$, Steel with Control)

Fabrics



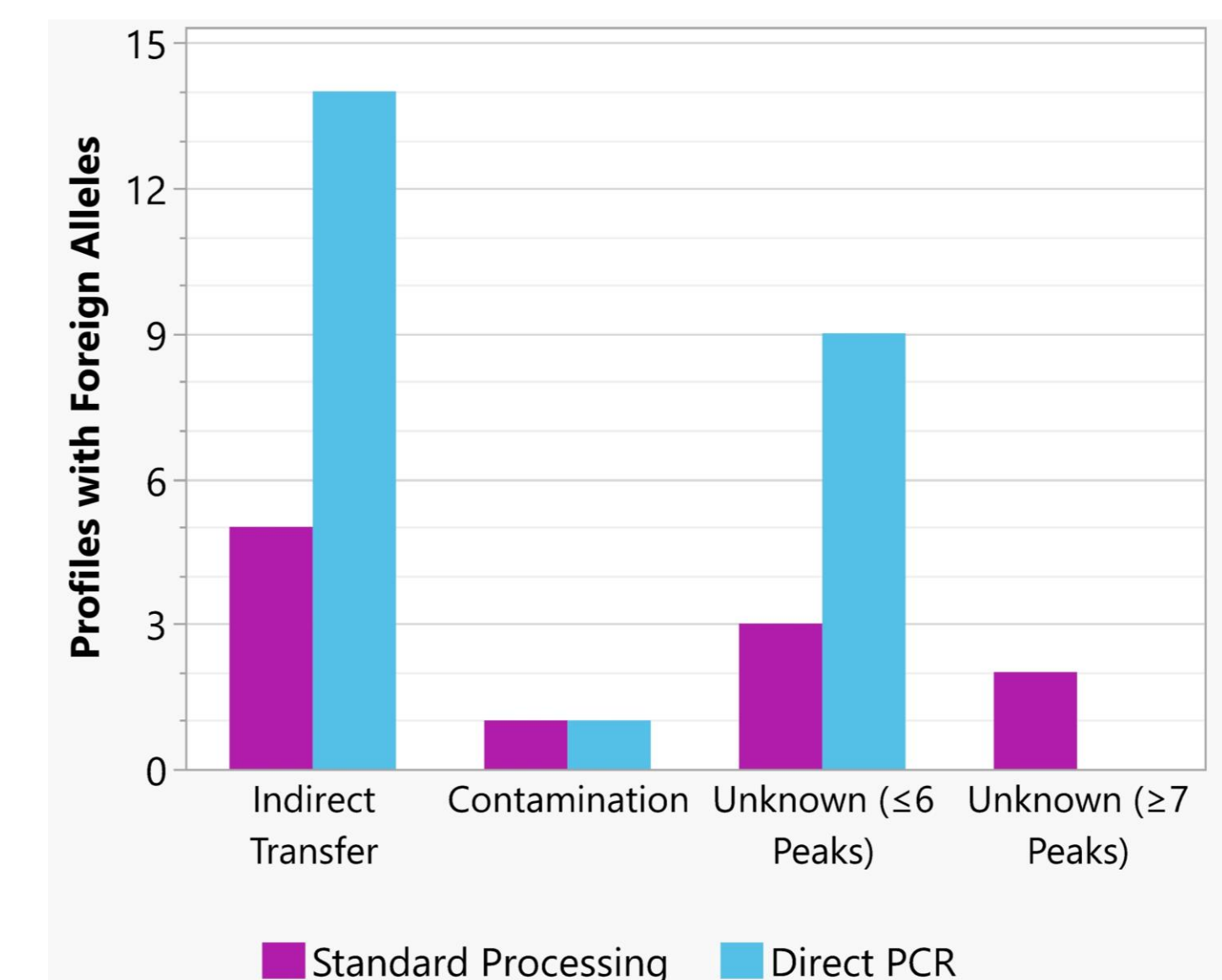
Non-Fabrics



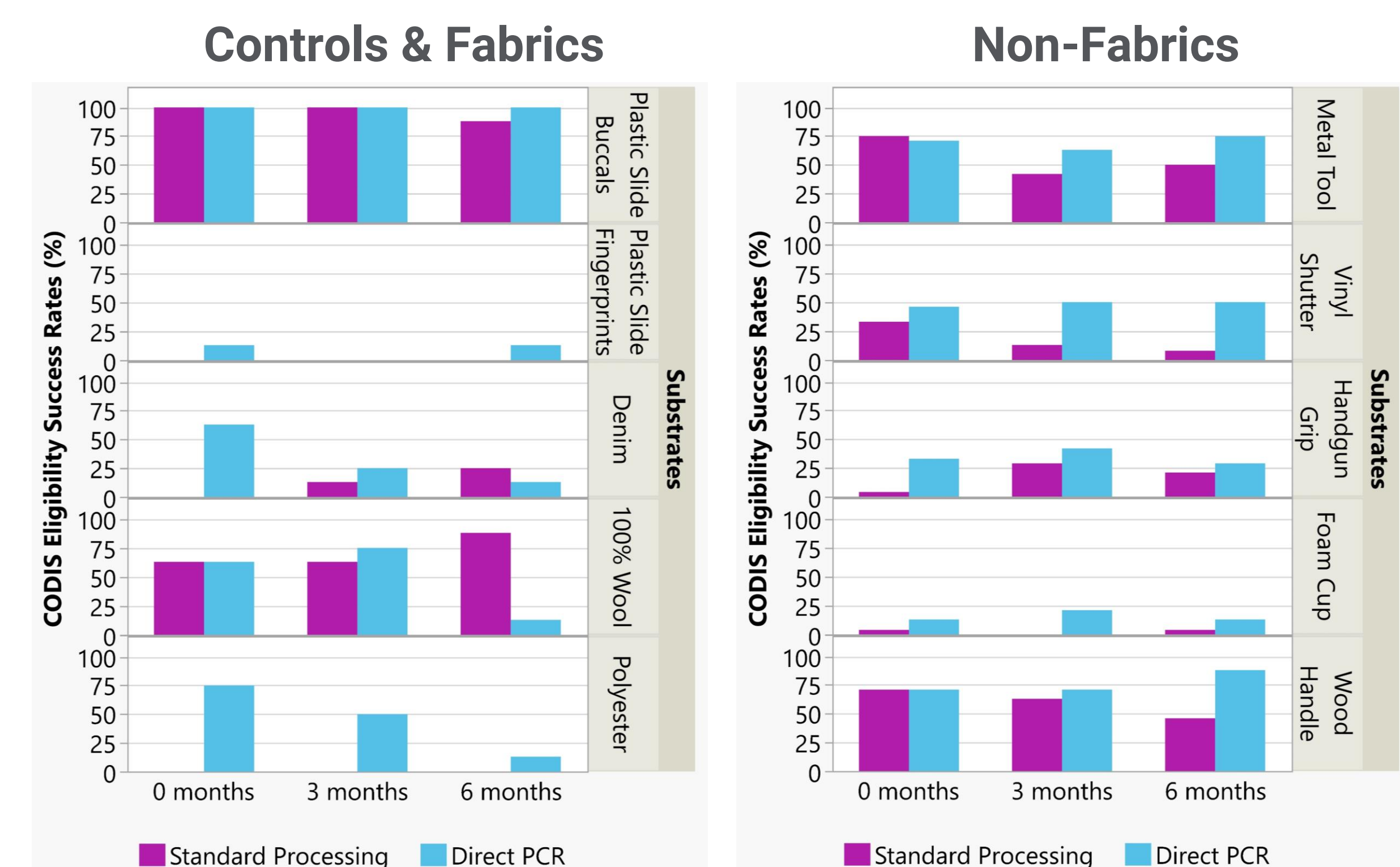
- Direct PCR was unsuccessful for concrete bricks and cartridge casings (data not shown).
 - Cartridge casings are known to present challenges for DNA¹.
 - Concrete contains minerals that may negatively affect PCR and CE.

Foreign Alleles

- Foreign alleles in 1.8% of standard processing samples & 3.9% of direct PCR samples
- Indirect transfer & contamination: foreign alleles consistent with known DNA profiles
- Unknown: foreign alleles could not be attributed to known DNA profiles



CODIS Eligibility



- Concrete bricks and cartridge casings did not produce CODIS-eligible profiles.

Conclusions

- Direct PCR results were comparable to or better than standard processing for all substrates and time points, excluding 100% wool at 6 months, concrete bricks, & cartridge casings.
- Direct PCR results from swabs and cuttings stored for 3 and 6 months did not significantly differ from those processed immediately, excluding polyester at 6 months.

References

- Montpetit S. Obtaining DNA from ammunition: A review. WIREs Forensic Sci 2020;2:e1352. doi: 10.1002/wfs2.1352.

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