

Comparison of GlobalFiler™ and PowerPlex® Fusion 6C for Direct PCR Amplification of Touch DNA Samples

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Introduction

- Direct polymerase chain reaction (PCR) amplification is a sample processing method in which a swab or substrate punch is added directly to an amplification reaction without prior extraction or quantification.
 - May improve DNA profiles from low-yield touch DNA samples¹.
 - Eliminates DNA loss that occurs during standard processing.
- The amount of DNA available for direct PCR can be affected by the method used for DNA sample collection.
 - The effectiveness of various collection methods is substrate specific.
 - Optimal direct PCR-compatible collection methods for each substrate were identified for further testing.
- DNA profiling results from low template samples can be affected by the STR multiplex kit used for amplification. These effects may be substrate or collection method specific.
- The main objective of this study was to compare STR profile results between touch DNA samples amplified via direct PCR with the GlobalFiler™ (GF) and PowerPlex® Fusion 6C (PPF6C) STR megaplex kits.

Touch DNA Sample Substrates



Methods

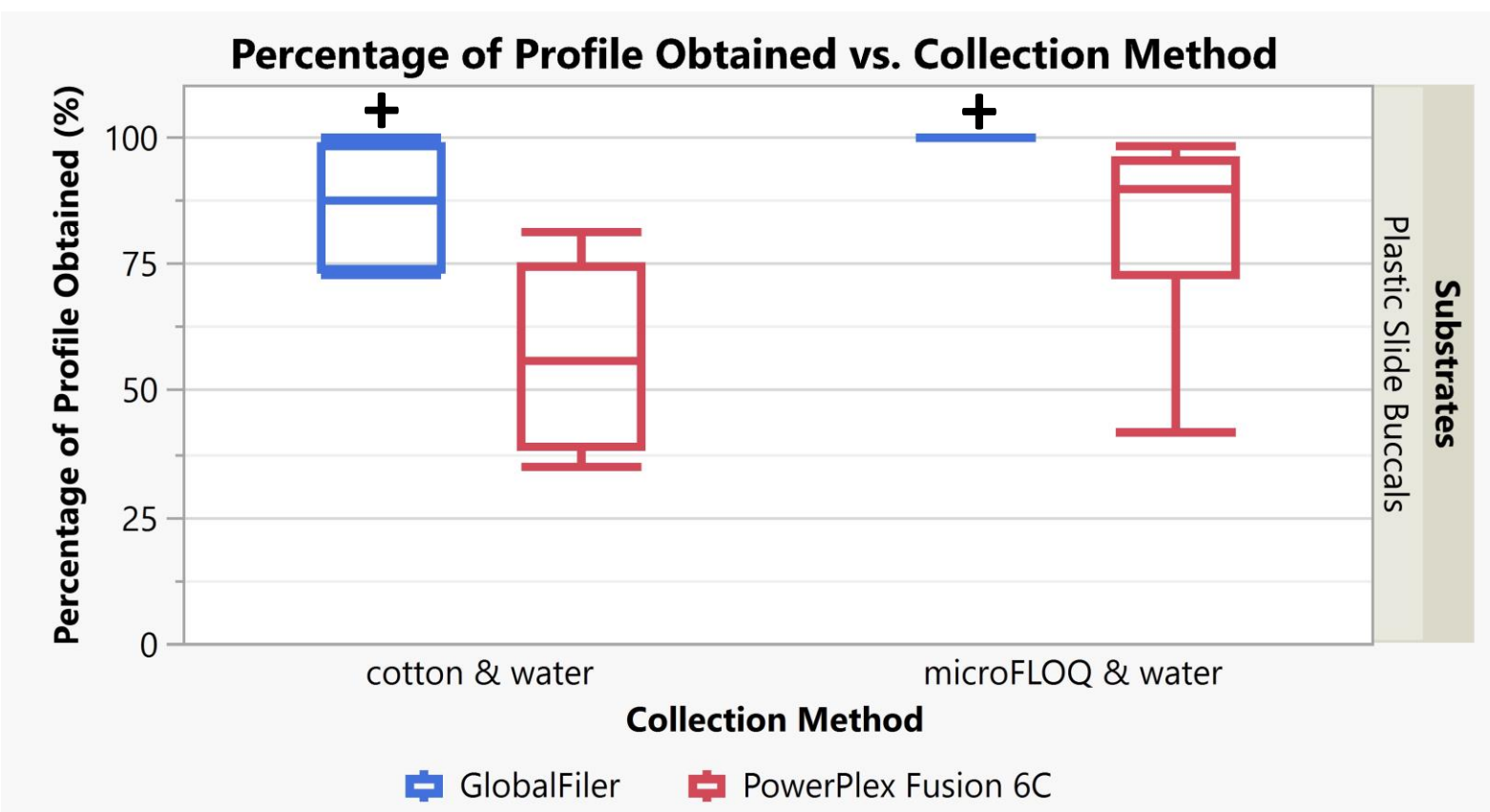
Collection Substrate	Diameter (mm)	Collection Method	Moistening Agent
Puritan® cotton swab	3.0	Swabbing	Sterile H ₂ O 0.1% Triton X None - dry
Copan microFLOQ® swab	1.2-2.0	Swabbing	Sterile H ₂ O 0.1% Triton X None - dry
Fabric	2.0	Cutting	None - dry

	Process	Method	
- Direct PCR - GF: 25 µL, 29 cycles - PPF6C: 25 µL, 29 cycles - Eight replicates per processing method, amp system, donor, and collection method (n = 720)	Amplification	GlobalFiler	PowerPlex Fusion 6C
	CE	3500xL Genetic Analyzer	
	Data analysis	GeneMapper ID-X® v1.5	
	Analytical threshold	125 RFU	100 RFU
	Stochastic threshold	600 RFU	500 RFU
	Evaluation metric	Percentage of profile obtained (% profile)	

Controls

Buccal Cell Spots

- The amount of touch DNA deposited when handling objects is inherently variable², even under controlled conditions.
- Because of this, samples containing controlled quantities of DNA (1.0 ng) were created by spotting buccal cells eluted in 1X PBS on plastic slides.

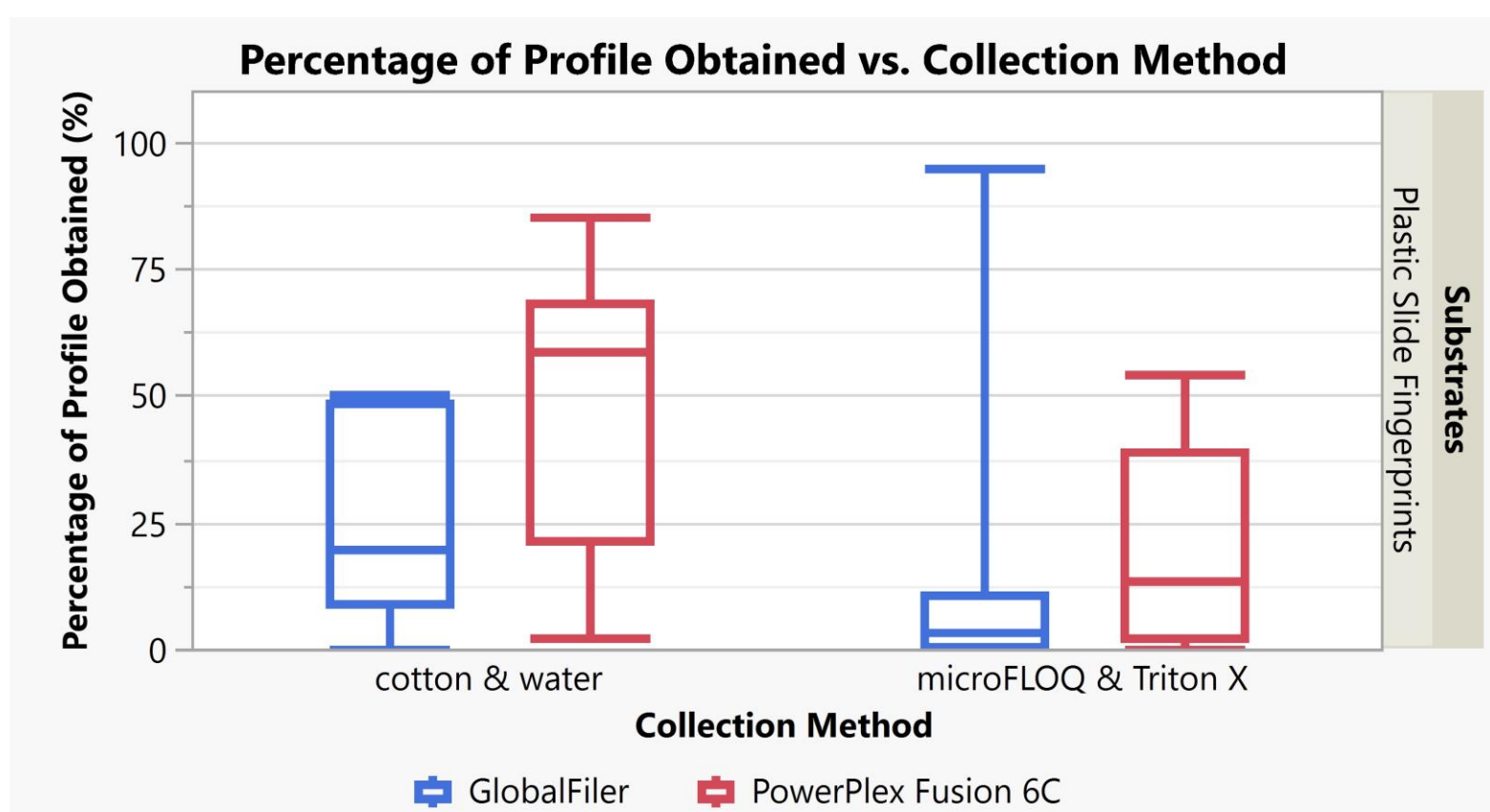


+ Significantly higher % profile from GF (p < 0.05, Wilcoxon Test)

- However, eluted buccal epithelial cells cannot perfectly mimic touch DNA deposits.
 - No cell-free DNA component
 - No oils, bacteria, or keratinized, anucleate skin cells
 - No information about the rates of indirect transfer
- These components of touch DNA deposits may impact direct PCR results.

Fingerprints

- Fingerprints were deposited on plastic slides under controlled conditions as a baseline for actual touch DNA depositions.
 - Contact for 1 min, 100 g pressure, 2 h post hand washing

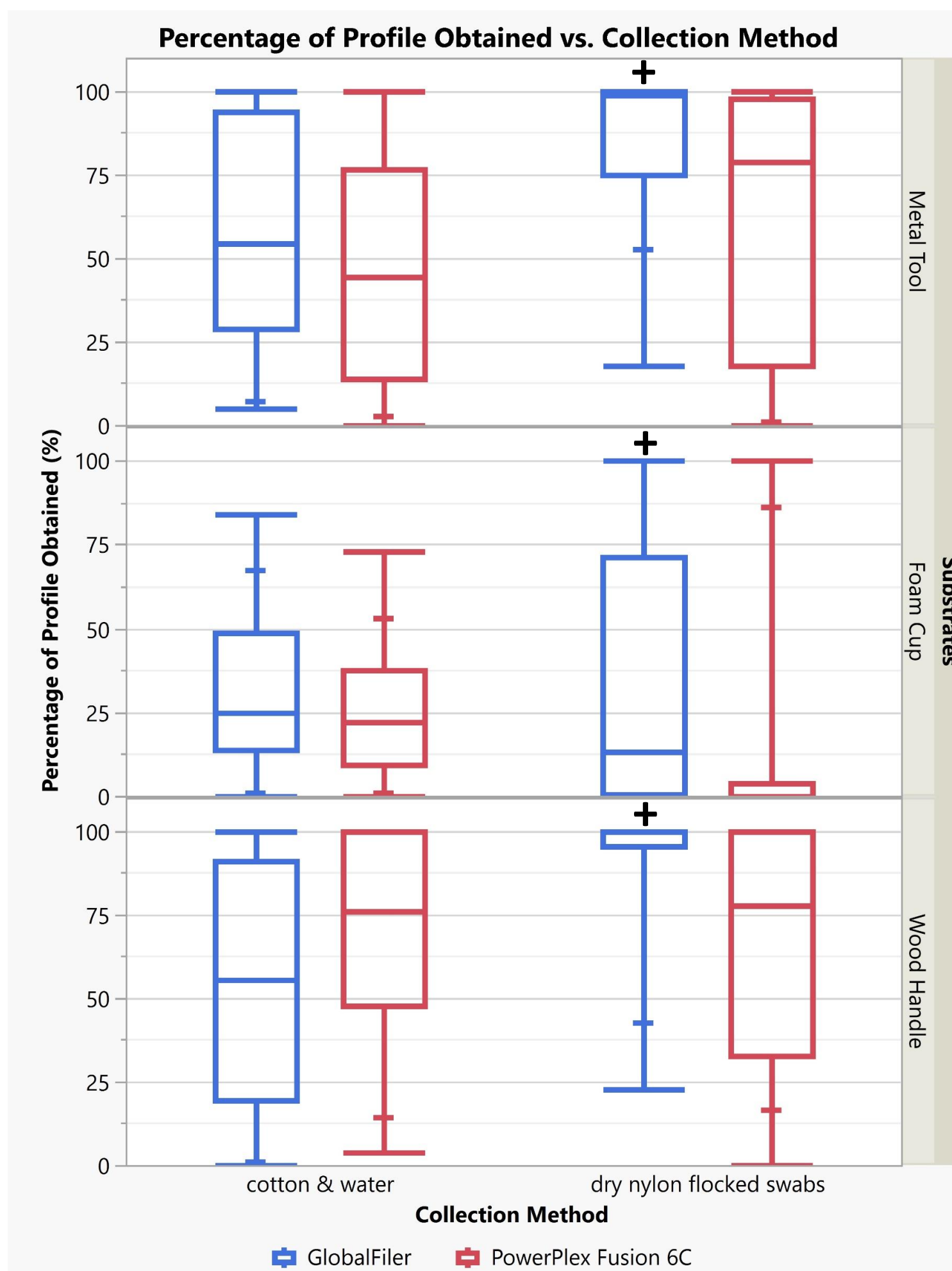


- Pros: contain all components of touch DNA deposits not included in buccal cell controls that could affect collection and direct PCR success; known/observable area of contact
- Cons: cannot standardize the amount of DNA deposited even under controlled conditions

Results & Discussion

Percent Profile GF vs PPF6C Non-Fabrics

- The optimal collection method to be used with direct PCR was identified for each substrate in previous work. Collection with cotton & water was selected for comparison as a control method as it is commonly used in forensics.
- The % profile obtained with GF and PPF6C direct amplification was compared for each substrate and collection method.



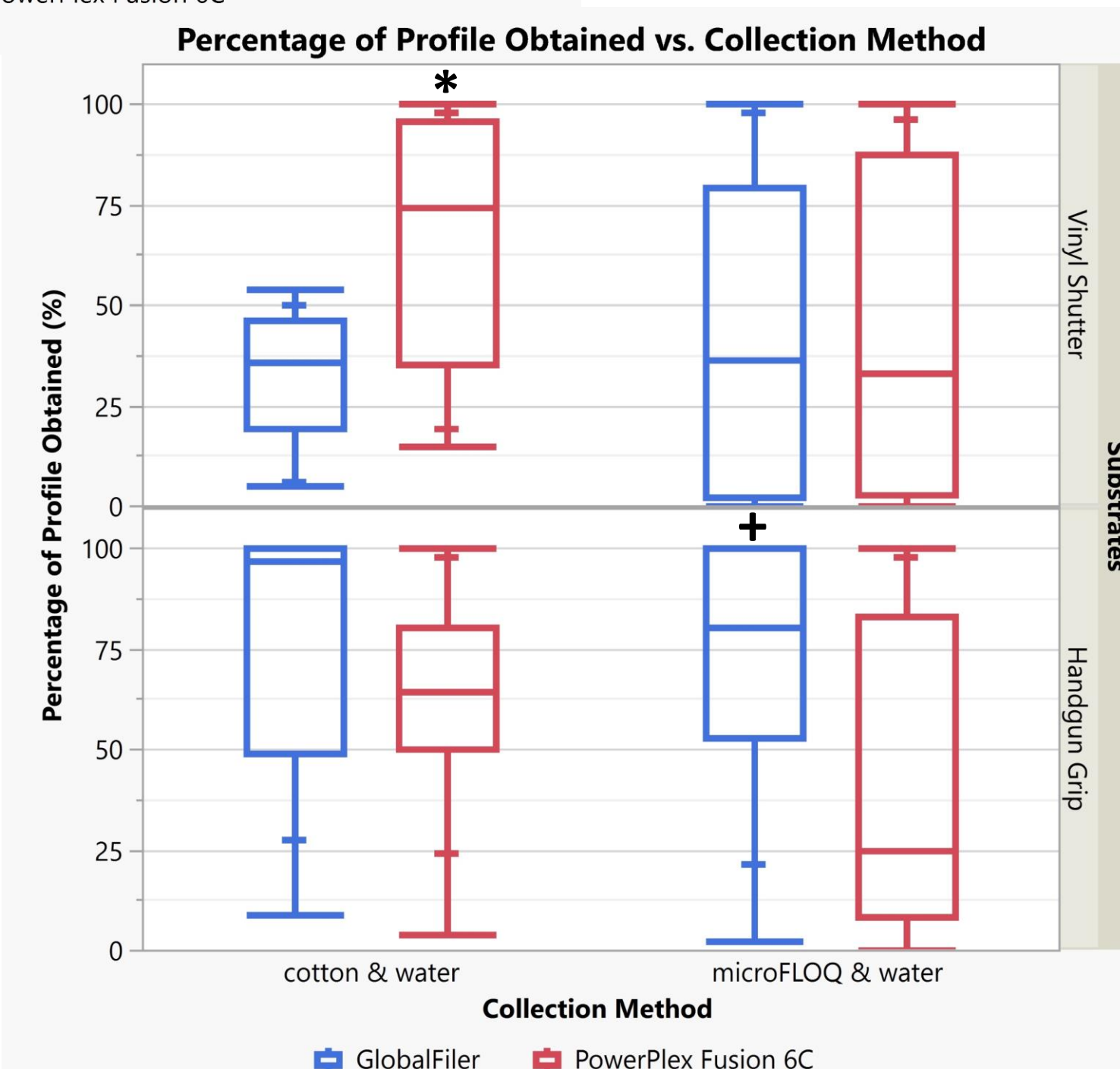
- No significant differences were found between GF and PPF6C amplification with the cotton & water collection method in 5 of 6 substrates

- The % profiles were significantly greater when samples were collected from vinyl shutters with cotton & water and amplified with PPF6C compared to GF

- GF resulted in significantly greater % profiles obtained from 5 of 6 collection substrates when microFLOQ swabs were used compared to PPF6C

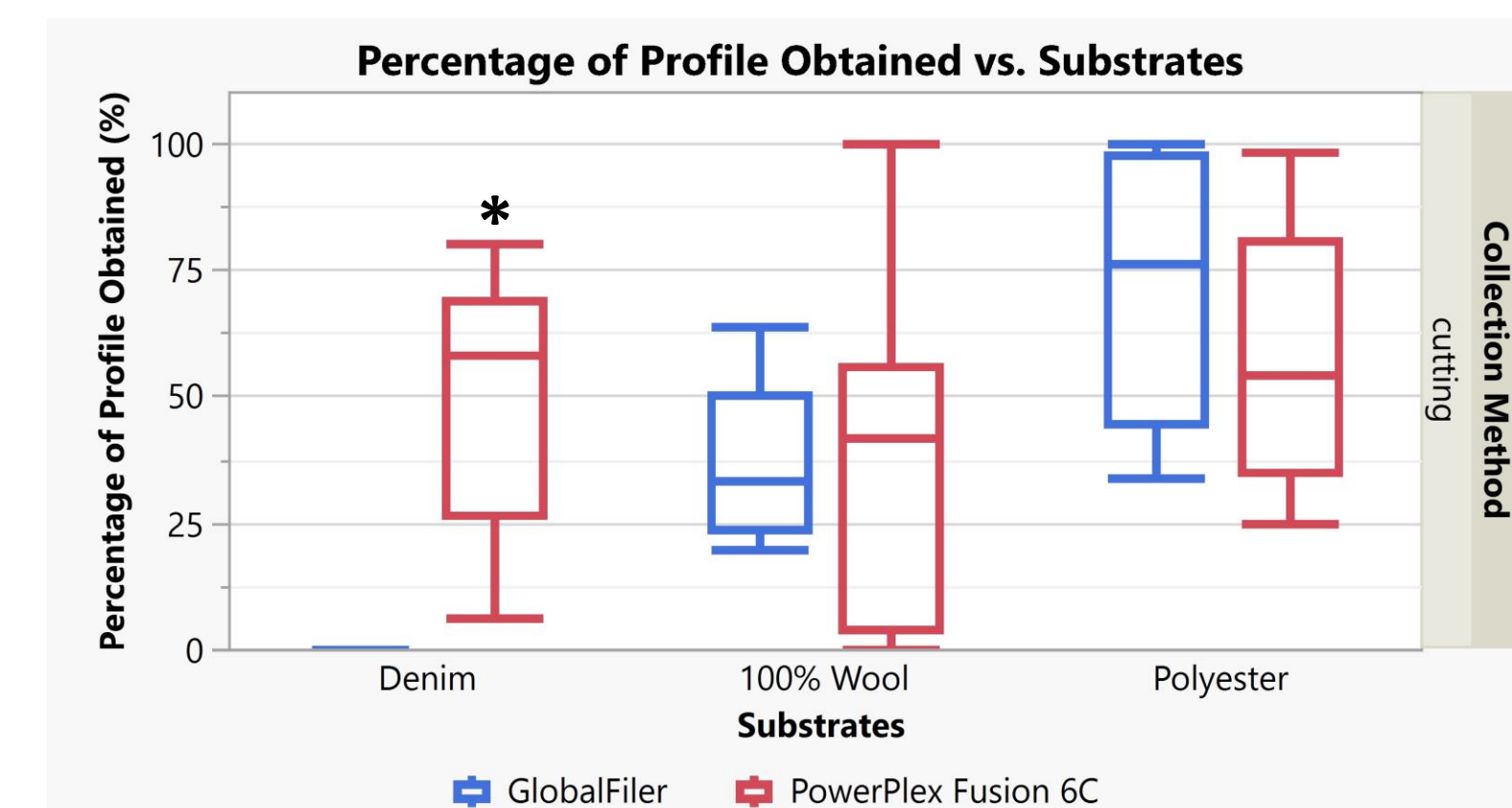
* Significantly higher % profile from PPF6C (p < 0.05, Wilcoxon Test)
+ Significantly higher % profile from GF (p < 0.05, Wilcoxon Test)

- Direct PCR from concrete bricks and fired cartridge casings was not successful with either GF or PPF6C due to PCR inhibition
- Cartridge casings are known to present challenges for DNA³
- Concrete contains minerals that may negatively affect both PCR and CE



Percent Profile GF vs PPF6C Fabrics

- Denim fabric was successfully amplified via direct PCR with the PPF6C kit but not the GF kit.
- Results from Wool and Polyester cuttings were comparable between GF and PPF6C.

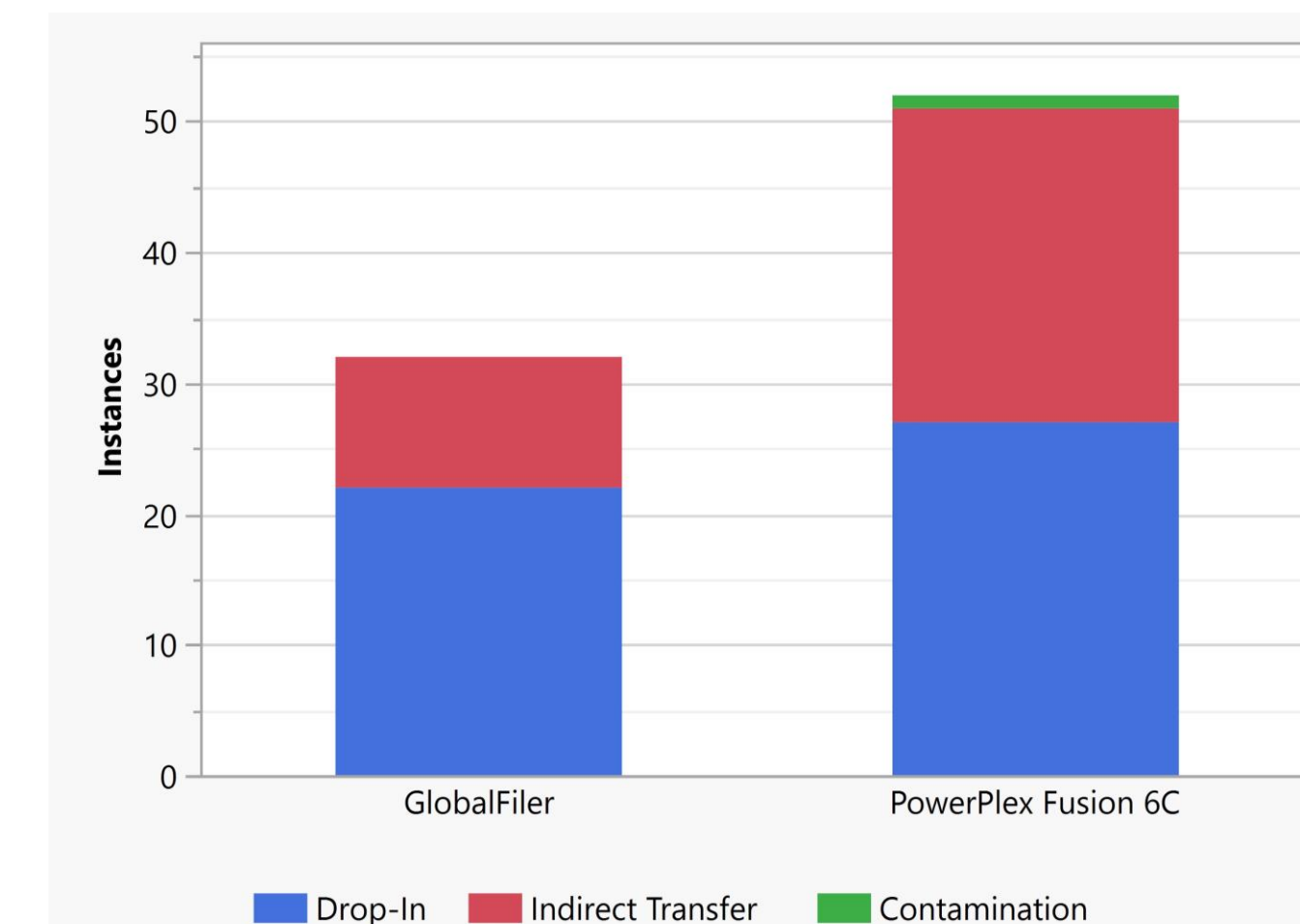


* Significantly higher % profile from PPF6C (p < 0.05, Wilcoxon test)

- PPF6C appeared to have greater resistance to the indigo dye present in the denim fabric⁴ when compared to GF.
- Melanin from the wool appeared to adversely affect direct PCR with both amplification kits⁵.

Allelic Drop-In & Indirect DNA Transfer

- Non-donor alleles were detected in 8.9% of GF and 14.4% of PPF6C direct PCR samples.



- Indirect transfer and contamination occurred when non-donor alleles were consistent with known DNA profiles. They were distinguished by context.
- Drop-in occurred when non-donor alleles could not be attributed to a known DNA profile. Most drop-in events involved ≤3 alleles (90% GF and 88% PPF6C).
- Drop-in rates were similar with both kits (2.8% GF, 6.7% PPF6C). These events highlight the sensitivity of modern STR multiplex kits.
- Practitioners should be aware that mixture deconvolution may be beneficial.

Conclusions

- In general, direct PCR of samples collected with cotton swabs & water provided comparable results between GF and PPF6C.
 - Only samples collected from vinyl shutters resulted in significantly higher percent profiles when PPF6C was used.
- In most cases, GF performed better than PPF6C when amplifying samples collected with microFLOQ swabs.
- Direct PCR of concrete bricks and fired cartridge casings was not successful with either kit.
- PPF6C successfully amplified denim cuttings, indicating it may have greater resistance to some PCR inhibitors, including indigo dye from denim, compared to GF.
- Direct PCR with PPF6C and GF was successful for many substrates without using specialized amplification kits or modifying validated amplification SOPs.
- Regardless of the collection and processing methods used, all touch DNA samples contain highly variable quantities of DNA.

Future Research

- Evaluation of the effectiveness of direct PCR on swabs stored after collection would be valuable as forensic DNA evidence is not always processed immediately after collection.
- Re-sampling of areas previously consumed for direct PCR to determine whether touch DNA remains for re-testing.
- PCR reaction optimization and additional post-PCR cleanup steps to improve the direct PCR results obtained from denim, wool, concrete bricks, and fired cartridge casings.

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