Comparative Analysis of DNA Extraction Protocols and the Dynamics of DNA and RNA Transfer

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INTRODUCTION AND AIMS

Deoxyribonucleic acid (DNA) profiling represents a fundamental tool in forensic investigations involving illicit activities. Understanding the mechanisms underlying DNA transfer is critical for interpreting the activity level and contextual relevance of recovered genetic material. The occurrence of secondary (indirect) transfer highlights the potential for detecting an individual's DNA on an item without direct physical contact (1). Furthermore, Ribonucleic acid (RNA) analysis has emerged as a promising approach for identifying the cellular origin of biological material and enhancing the resolution of the association between a suspect and forensic evidence (2).

This study had two primary objectives: a) assess and compare the efficiency of extraction protocols—*Chelex* Vs. a co-extraction method enabling the simultaneous isolation of DNA and RNA—; and b) apply the co-extraction protocol to investigate the dynamics of secondary (indirect) transfer of DNA and RNA from the interior surface of a previously handled bag to a plastic wrapping.

MATERIALS AND METHODS

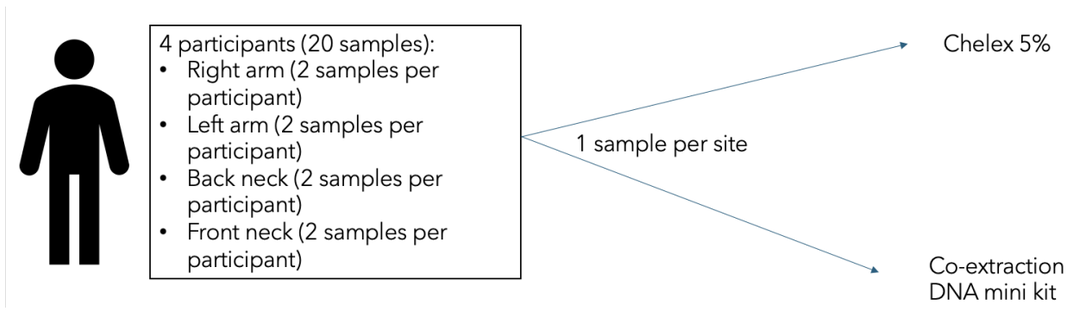

Fig. 1: Extraction test. Following DNA extraction, samples were quantified using the PowerQuant System (Promega), amplified with the PowerPlex 6C System (Promega), and analysed by fragment analysis.

Fig. 2: Indirect Transfer Study: **Step 1:** Two zip-lock bags were placed inside a larger bag and left undisturbed for 24 hours. **Step 2:** Following this period, samples were collected—one from the surface of a zip-lock bag and one from the interior surface of the larger bag.

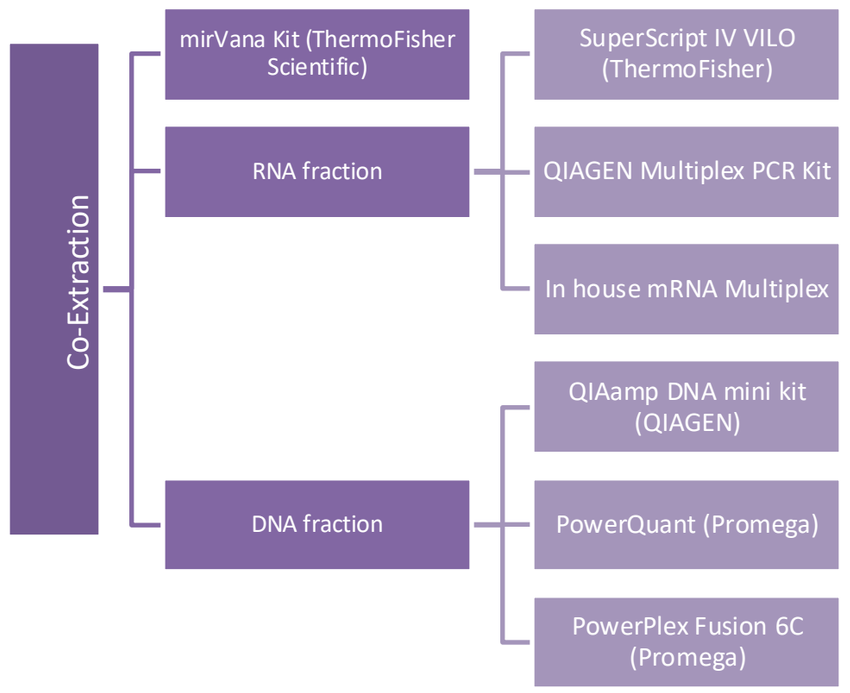

Fig. 3. Co-extraction Protocol Flowchart.

RESULTS

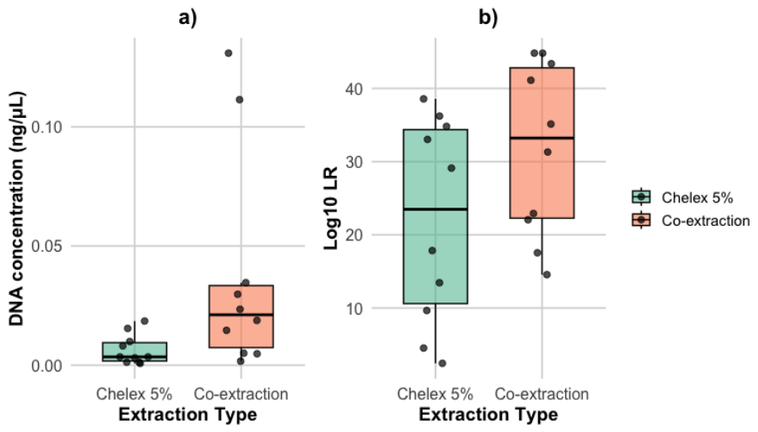

Fig. 4. Boxplot showing DNA concentrations for Chelex 5% (ng/µL in 200 µL, green) and Co-extraction (ng/µL in 100 µL, orange), along with the corresponding $Log_{10}LR$ values for both methods.

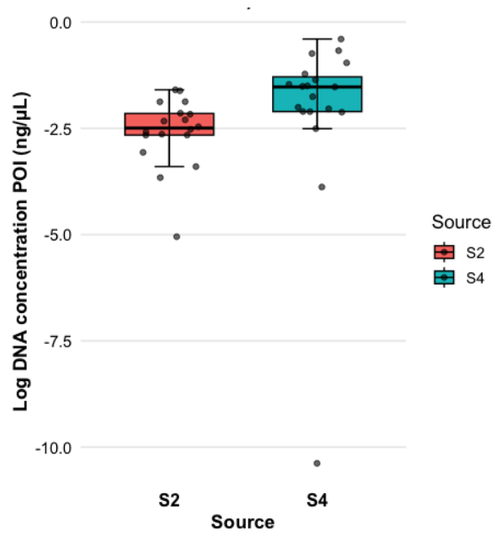

Fig. 5. Boxplots comparing DNA concentrations Log_{10}-transformed, ng/µL matching the person of interest (POI) in samples S2 (zip-lock bag, coral) and S4 (interior of the bag, blue)

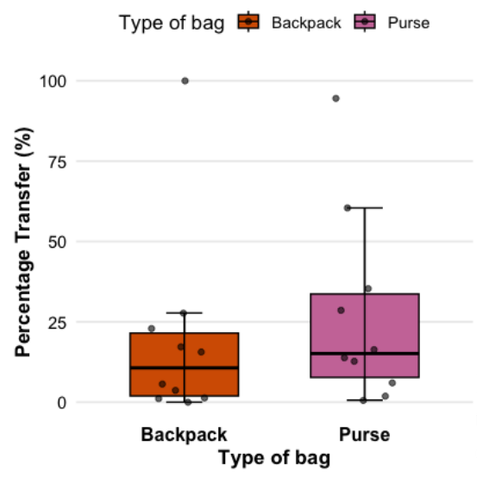

Fig. 6. Boxplots showing the percentage of DNA transferred from the interior of the bag (S4) to the corresponding zip-lock collection bag (S2), grouped by bag type—backpack (orange) and purse (pink).

Table 1: Synthesis of RNA Profiling Findings.

Category	Key Finding / Result	Percentage (%) / Sample Count (N)	Associated Markers
Cellular Material	Presence confirmed	82.5% (N=33)	Two housekeeping genes detected
	Negative	17.5% (N=7)	housekeeping genes not detected)
Specific Blood Detection	Blood identified	2.5% (N=1)	HBB, CD93
Sex Marker Detection	Total samples with sex markers	N=7	-
	Female marker detected	10% (N=4)	XIST
	Male marker detected	7.5% (N=3)	RPS4Y1
Sporadic Body Fluid Detections	Total samples with sporadic fluid detection	20% (N=7)	
	Sporadic Saliva detected	N=2	STATH
	Sporadic Blood & Mucosa detected	N=1	CD93, MUC4
	Sporadic Blood (single marker) detected	N=1	HBB
	Sporadic Multi-Fluid (Blood, Saliva, Menstrual Blood) detected	N=1	HBB, STATH, MMP11

CONCLUSION

Our findings demonstrate that the co-extraction method is more effective for recovering DNA from low-template. Moreover, our results confirm the feasibility of detecting both DNA and RNA from secondary transfer events between distinct surfaces. Higher quantity of DNA retrieved from backpacks in comparison with purses. mRNA profiles, 1 sample showed presence of blood and the rest only sporadic detection of body-fluids.

REFERENCES

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