



Integrating Multi-Omics for the Temporal Profiling of Human Biological Traces to Improve Time Since Deposition Estimation

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PROBLEM

Time since Deposition (TsD) is the interval between the deposition of a biological fluid at a crime scene and its collection and analysis. Estimating this interval is crucial for crime-scene reconstruction and interpretation of biological evidence.

Current forensic methods can reliably identify the **donor** and the **type of biological fluid**, but they do not provide robust information on **when** the trace was deposited. This limits interpretation when the temporal relevance of a trace is central. TsD estimation can support the transition from **source-level** to **activity-level** interpretation [1–3].

However, current approaches remain limited, as most studies focus on dried blood, while saliva and semen are still underexplored, and many rely on single-omics analyses.

Since forensic traces are often **limited, degraded and non-repeatable**, integrated workflows are needed to maximise molecular information from a single trace and improve TsD estimation across different fluids and deposition conditions [4–5].

INNOVATION

This project applies an **integrated multi-omics approach** to study the molecular ageing of **blood, saliva and semen** for **Time since Deposition (TsD)** estimation.

The workflow aims to recover **proteins, metabolites and lipids** from the **same trace** using a unified **co-extraction protocol**. This allows multiple molecular layers to be analysed in parallel, instead of processing each class separately.

By combining these complementary profiles, the project will identify **molecular ageing signatures** linked to trace deposition over time.

These signatures will then be integrated into a **multi-omics model** to support more robust and reproducible TsD estimation.

Standardised **Copan swab-based systems** will be incorporated to improve recovery, reproducibility and compatibility with forensic workflows.

The Forens-OMICS Approach

Integrated workflow for multi-omic analysis and time since deposition (TsD) estimation

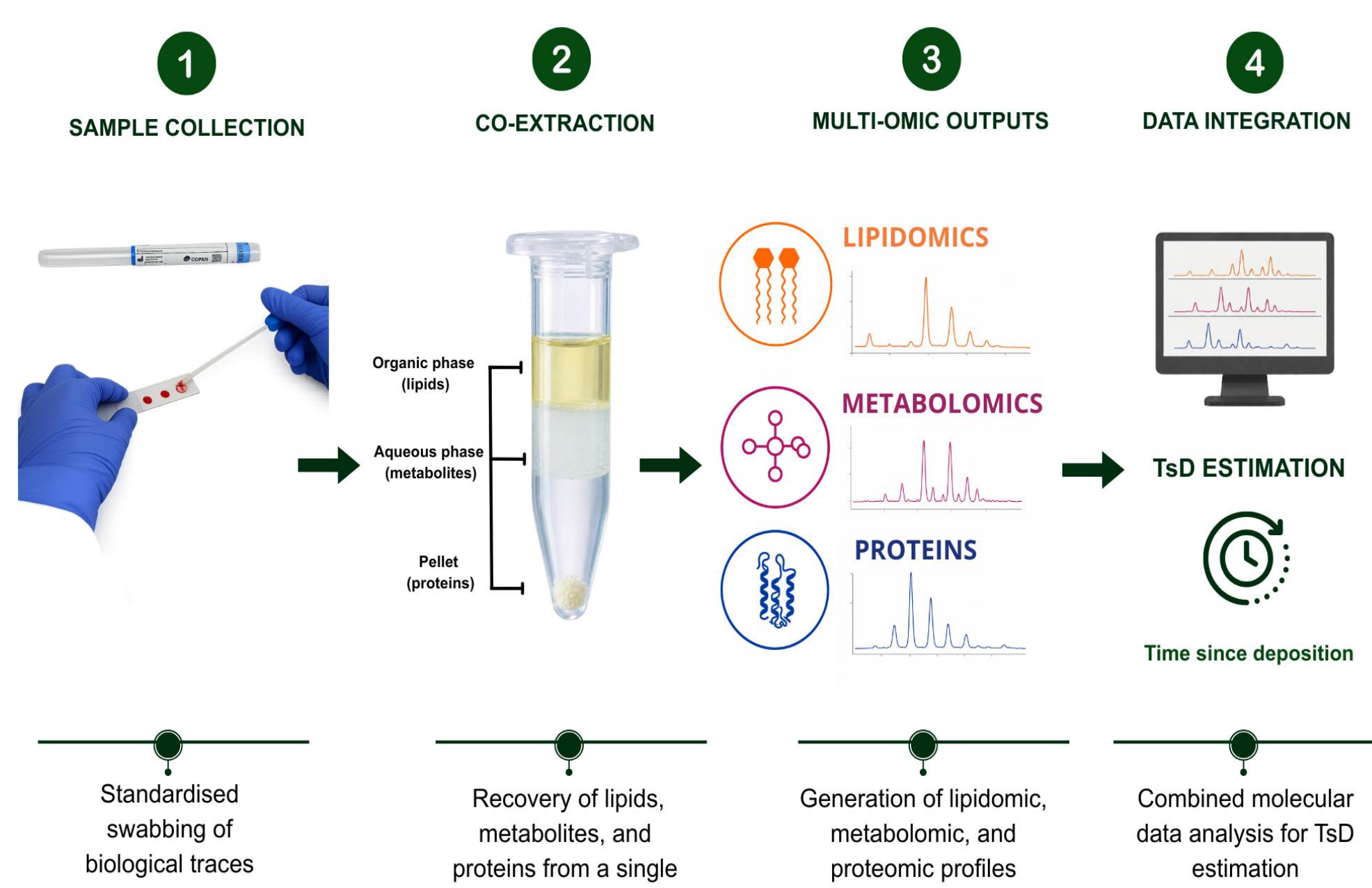


Figure 1. Schematic overview of the Forens-OMICS workflow for Time since Deposition (TsD) estimation.

STUDY DESIGN & WORKFLOW

The study includes three biological fluids: **blood, saliva and semen**, collected from **six donors per fluid** to account for biological variability.

For each donor, **20 µL** of fluid are deposited on **eight forensic surfaces**: four non-porous and four porous, selected to evaluate the effect of surface type on drying, recovery and molecular degradation. Samples are collected at **ten time points**, from early post-drying to **one year after deposition**, to monitor short- and long-term molecular changes.

Fresh samples are first analysed at **T0** to define the baseline molecular profile and identify candidate biomarkers for **TsD estimation**.

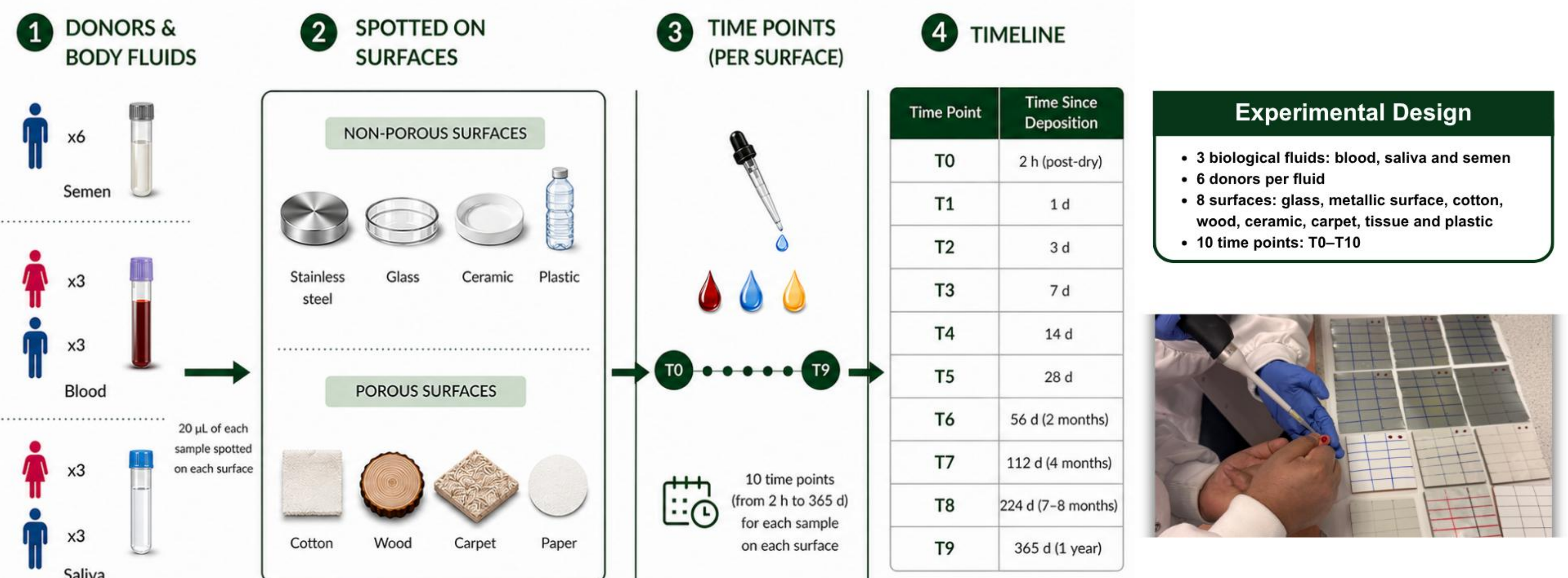


FIGURE 2. The Forens OMICS project investigates three biological fluids (blood, saliva and semen) on eight surfaces (four non-porous and four porous). Samples from 6 donors per fluid will be analysed at 10 time points (2 h to 365 days) to assess multi-molecular degradation and persistence over time.

MULTI-OMICS WORKFLOW & MOLECULAR RECOVERY

Five extraction protocols were tested for simultaneous recovery of metabolites, lipids and proteins from a single 20 µL trace. The MTBE-based biphasic extraction (**Matyash method**) showed the best overall performance.



Figure 3: Optimised MTBE-based biphasic extraction enabled simultaneous recovery of lipids, metabolites and proteins from a single 20 µL trace. Representative untargeted GC-MS profiles show distinct molecular signatures across blood, saliva and semen, while protein quantification confirmed sufficient recovery for downstream S-Trap and LC-MS/MS analysis.

IMPACT

By combining multiple omics layers from the **same biological trace**, this project aims to identify biomarker panels with consistent temporal patterns across **different fluids, donors, surfaces and deposition times**.

This approach maximises the molecular information obtained from **small, limited or degraded forensic samples**, overcoming the current limitation of having to prioritise one analytical method when sample quantity is low. Instead, proteins, metabolites and lipids can be evaluated together to provide a more complete picture of trace ageing.

The resulting multi-omics signatures will support the development of **quantitative and evidence-based TsD prediction models**, strengthening crime-scene reconstruction, improving activity-level interpretation and increasing the evidential value of biological traces.

REFERENCES

- [1] 10.1002/dta.3480; [2] 10.3389/fmicb.2021.647933; [3] 10.1016/j.fsigs.2019.10.028; [4] 10.1016/j.fsigen.2023.103845; [5] 10.1021/acs.analchem.4c04421