Evaluation of Time Dependent Protein Signatures in Dried Blood

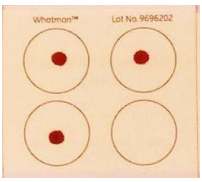
Thalassa S.E. Valkenburg[a*], Paul S. Monks[b], Hitesh C. Pandya[c], Garry L. Corthals[d]

[a]University of Leicester, INTREPID Forensics, United Kingdom, *tsev1@le.ac.uk
[b]University of Leicester, Department of Chemistry, United Kingdom
[c]University of Leicester, Department of Immunity, Infection & Inflammation, United Kingdom
[d]University of Amsterdam, Van 't Hoff Institute for Molecular Sciences, the Netherlands

Dried Blood Spots

Dried Blood Spots (DBS) were used for the time first in 1963 by Dr Guthrie[1]. He used them to detect disorders in neonates.

- First application: screening for inborn disorders in neonates.
- New applications: a wide range has evolved[2].
- Forensic applications: of special interest for this project with most current applications focussing on chemical analyses[2].
- This study: evaluating time dependent signatures of dried blood proteins.



Proteomics Approach

- DBS: 10 µl capillary blood spotted on human ID bloodstain cards.
- Time: every day evaluation, up to 1 week.
- Ageing conditions: in dark with circulating air at room temperature.
- Amount of samples: triplicate samples per time point from 6 healthy volunteers.
- DBS treatment: extraction of blood from cards with ammonium bicarbonate, denaturation, reduction, alkylation, overnight trypsin digestion, solid phase extraction (SPE) and evaporation to dryness.
- Sample analysis: RP-HPLC on in-house packed 75 µm columns, tandem MS in positive ion mode on 2+, 3+ and 4+ ions in the range from 400 to 1250 a.m.u. and identification using Protein Pilot.

Preliminary Results

- Up to 185 proteins and 2087 peptide signatures have been identified in the ageing DBS with 1% False Discovery Rate (FDR). A slight increase in the number of different proteins and peptides can be observed from spots sampled at day 0 until samples aged up to 1 week (Fig 1).

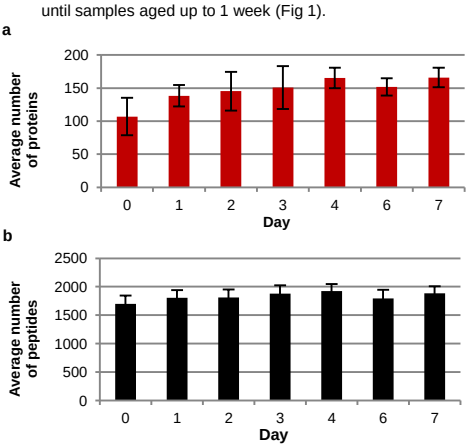


Figure 1: Amount of proteins (a) and peptides (b) over time.

- A closer look of the amount of proteins detected on the consecutive days reveals that 126 proteins overlap for all days aged up to one week (Fig 2).

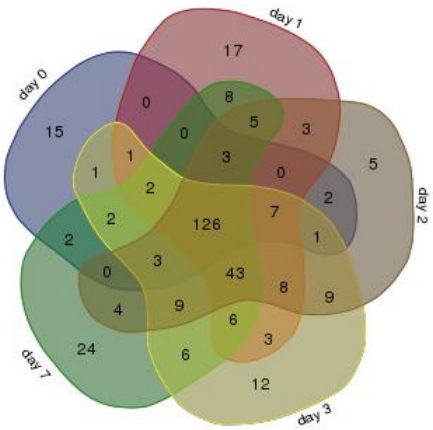


Figure 2: Venn-diagram showing overlapping and unique proteins.

Conclusion and Future Work

- A start has been made identifying time dependent signatures of dried blood proteins from six donors.
- Quantification of de identified proteins over time will be performed in the near future.

Once a selection of time dependent markers is established, a similar approach may be used as introduced by Borchers *et al.*[3]. This group detected and quantified 97 proteins in a targeted manner using multiple reaction monitoring (MRM), but eliminated instable proteins for medical purposes. This project will focus on proteins varying in quantity over time for forensic purposes.

References

1. Guthrie R. and Susi A. (1963). A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. *Paediatrics*.
2. Stove C. P., Ingels A. S. M., De Kesel P. M. and Lambert W. E. (2012). Dried blood spots in toxicology: from the cradle to the grave?. *Critical reviews in toxicology*.
3. Chambers A.G., Percy A.J., Yang J. and Borchers C.H. (2015). Multiple Reaction Monitoring Enables Precise Quantification of 97 Proteins in Dried Blood Spots. *Molecular & Cellular Proteomics*.

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