B5 Evaluation Surface Effect of Bloodstains on Various Absorbent and Non-Absorbent Surfaces Using a Metabolomic Approach

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Learning Overview: After attending this presentation, attendees will understand that the recovery rate of components in the bloodstain varies depending on the surface of the bloodstain. Through this study, when collecting evidence in the field, it will be possible to refer to the priority of collecting bloodstain surfaces for a more accurate analysis of bloodstain components.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by suggesting a preliminary guide that provides a reference for the collection priority of bloodstain surfaces when analyzing bloodstain metabolites.

Bloodstains found at crime scenes contain immense information about the crime; thus, studies involving the analysis of small molecules in bloodstains have been conducted. However, most of these studies have not accounted for the difference in the results of small molecule analysis due to the surface of bloodstains. Therefore, it is necessary to observe the difference in components of bloodstains, which are extracted from different surfaces known as the "surface effect." The purpose of this study is to identify the surface with the least effect and interference on the analysis of small molecules in bloodstains.

To evaluate the "surface effect," this study prepared bloodstains on seven surfaces, including both absorbent (filter paper and mixed cotton) and non-absorbent surfaces (glass, tile, stainless steel, vinyl flooring, and wooden flooring). After collection of the bloodstain samples, metabolites were extracted from the bloodstains, then global small molecule analysis was performed by High-Performance Liquid Chromatography/Tandem Mass Spectrometry (HPLC/MS/MS). The multivariate analysis, such as heat map, correlation analysis, and Principal Component Analysis (PCA) was performed by Mass Profiler Professional (MPP) and MetaboAnalyst. Euclidean and Pearson distance metrics were used for heat map and correlation analysis, respectively. The common Molecular Features (MFs) were matched to the Agilent® METLIN PCDL B.08.00 database at the MS level using Agilent® MassHunter Qualitative Analysis.

To determine the ranking of the seven surfaces in the order of their similarity with blood, this study used three indicators: (1) count recovery rate (%) of MFs; (2) the number of MFs extracted from the surface without bloodstains: and (3) the difference in the abundance recovery rate (%) of MFs. The lower the sum of the rankings of all the results of each surface, the higher the ranking of that surface. Thus, based on a combined evaluation, this study found that the non-absorbent surfaces ranked better than the absorbent surfaces; wooden flooring was ranked as the most efficient surface, followed by stainless steel, vinyl flooring, glass, tile, filter paper, and mixed cotton. Additionally, this study confirmed the correlation between each surface and blood through multivariate analysis. Subsequently, non-absorbent surfaces tend to cluster more closely with the blood than the absorbent surfaces. This study will help in improving accuracy of small molecule analysis for bloodstain collection in the field in forensic science.

Bloodstain, Surface Effect, Metabolomics