B219 Thanatochemistry at the Crime Scene: A Microfluidic Paper-Based Device for Ammonium Analysis in the Vitreous Humor

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Learning Overview: After attending this presentation, attendees will be informed about the advantages of the use of paper-based microfluidic technology for inferring the Postmortem Interval (PMI).

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing a new tool for estimating the time since death. Thanks to the low cost and portability of the developed devices, this method has the potential of offering a real breakthrough at the crime scene, offering, for the first time, the possibility of a rapid and objective estimation of the time since death after the equilibration of the body temperature with the environment (approximately 18–20 hours).

The estimation of the time since death is a crucial question that forensic experts need to answer directly at the crime scene to effectively address the investigations.

Notwithstanding a large number of studies regarding the PMI estimation, this problem is still faced using body examination. To obtain more accurate and objective data, after the first 20 hours during which body cooling is typically used, most of the literature is based on the analysis of the postmortem changes occurring in the vitreous humor, such as potassium estimation. Recently, the correlation between the concentration of the ammonium ion in the vitreous humor and the time since death was demonstrated.[1] Although the analytical methods offer sensitivity and specificity, at the moment, thanatochemistry analyses require the application of expensive and complex separative instruments.

Since the first introduction by Whitesides et al., the use of Paper-Based Microfluidic Devices (µPADs) for the development of chemical sensors has been extensively reported.[2,3]. Among several approaches for producing µPADs, the use of commercial wax printers proved to be inexpensive and straightforward in the fabrication process. The procedure is based on two steps: (1) patterning chromatography paper into hydrophilic channels by fabricating hydrophobic barriers, and (2) the addition of the reagent to the hydrophilic portion of the paper support. The sample is driven through the reagent zone as results of the wicking capacity of the paper without external assistance. This paper-based technology proved to be rapid and easy-to-use. This approach does not require highly qualified personnel nor expensive instrumentation. Also, it can be performed onsite, also enabling a prompt analytical response in less-equipped environments. The advantages of µPADs have provided forensic science with reliable tools to face different forensic issues.

On the grounds of the above considerations, the goal of this presentation is to show the use of a low-cost device able to provide a rapid and sensitive colorimetric detection of the ammonium in vitreous humor. The developed procedure is conceived to obtain a first-line response for inferring the PMI before samples are sent to an equipped laboratory for further analysis. The preliminary results could be used to prioritize investigative directions.

The proposed microfluidic devices were designed as a single spot pattern. The test was based on a specific reaction for the detection of ammonium, which uses the Nessler's reagent. The vitreous humor sample is transferred without any treatment directly onto the hydrophilic portion of the paper, and a colorimetric reaction is developed in a few minutes. The color change is measured in terms of "Red, Green, Blue [RGB] distance" by using a simple and free application for smart phone cameras. The device was optimized to accurately and precisely quantify the ammonium concentration. The selectivity of the Nessler's reaction was tested toward the main vitreous humor components, and the stability over ten days was tested. The optimized device was used for the determination of ammonium ion in vitreous humor samples from forensic autopsies (n=25). The results obtained with the proposed method were compared with the data obtained with an instrumental analysis based on capillary electrophoresis.

In conclusion, the results of the use of a paper-based microfluidic approach and the advantages of the device as an additional tool to the traditional methods for an objective, although still presumptive, estimation of the time of death directly at the crime scene will be shown during the presentation.

Reference(s):

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3. D.M. Cate, J.A. Adkins, J. Mettakoonpitak, C.S. Henry. Recent developments in paper-based microfluidic devices. *Anal Chem.* 87 (2015) 19–41.

Microfluidic Paper-Based Devices, Postmortem Interval (PMI), Thanatochemistry at the Crime Scene

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