G50 The Application of Flow Cytometry as a Rapid Screening Method of Samples of Vitreous Humour to Avoid Miscalculation of the Postmortem Interval

Cristina Cordeiro, MD, Instituto Nacional de Medicina Legal, IP, Largo da Sé Nova, Coimbra, PORTUGAL; Rafael Seoane, PhD, Elena Lendoiro, BS, and María Sol Rodríguez-Calvo, PhD, Univ of Santiago de Compostela, Calle San Francisco, Santiago de Compostela, 15782, SPAIN; Duarte N. Vieira, PhD, MD, Univ of Coimbra, Rua Antonio Jose de Almeida, No 117, Coimbra, PORTUGAL; and José Ignácio Muñoz-Barús, PhD, Inst of Forensic Science, Univ of Santiago de Compostela, Calle San Francisco, Santiago de Compostela, SPAIN*

After attending this presentation, attendees will understand the confirmation and quantification of possible accidental contamination with blood of samples of vitreous humour by means of applying the flow cytometry method to the estimation of the Postmortem Interval (PMI).

This presentation will impact the forensic science community by demonstrating the susceptibility of vitreous humour to blood contamination and how this can affect PMI estimates. The determination of PMI is a critical issue in forensic science and methodologies based on the biochemistry of the vitreous humour can provide good results.

In the field of Legal Medicine, the correct determination of the time of death is of crucial importance due to its criminal and civil repercussions. In recent years, research into maximizing the precision and reliability of this estimation has been a recurring subject of investigation, and it is of utmost importance to have recourse to a method that can provide this information quickly and accurately. The best results for estimating time of death derive from the biochemistry of the Vitreous Humour (VH) and are based on examining the relationship between PMI and increases in K+ and hypoxanthine (Hx) levels. Latest reports in the literature suggest that the manner of death can also modify this relationship and computerized programs which take into account these variables have been published. However, contamination by causes not readily apparent, such as blood from the accidental puncture of blood vessels, can occur, and thus lead to an erroneous estimation of PMI.

Objective: The goal of this presentation is to confirm and quantify blood contamination in samples of vitreous humour by using the Flow Cytometry method which reveals how this contamination substantially alters the estimate of the PMI.

Method: Vitreous humour samples were obtained from fresh bodies, and artificially contaminated with known concentration of human blood (5µl of 10% blood in 300µl of HV) and serially diluted in FACSflow™ (Coulter) in order to determine the minimum amount of erythrocytes detectable and its relation to hypoxanthine quantification.

Samples were run in a Facscalibur cytometer (Coulter) at medium flow (12µl/min) and erythrocytes detected in an FCS vs. SSC density plot.

The detection was considered valid when the detected number of erythrocytes stood at the theoretical logarithm ± 0.3. Greater dilutions showed values closer to background noise.

Determination of Hx by LC-MS/MS: A Quattro Micro tandem mass spectrometer (Waters) was employed for the detection of Hx. Chromatographic separation was performed using an Atlantis T3 (2.1x100mm, 3µM) analytical column, working in gradient mode, with acetonitrile and ammonium acetate 10mM (pH=4.5) as mobile phase. Two precursor-product transitions per compound were monitored except for Internal Standard (IS).

Results and Discussion: This contamination noticeably affects the result, and thus emphasizes the importance of careful sample extraction. The cytometric method was able to detect erythrocytes in 1:1250 dilution of contaminated HV. This represents a presence of 6,000 erythrocytes per ml, much lower than that detectable by microscopic counting. This concentration is 0.0017µ| of blood in 1 millilitre of HV.

The sensitivity of the technique, its widespread distribution in the laboratories, and the speed of the results recommend it for the rapid screening of samples.

Postmortem Interval, Vitreous Humour, Flow Cytometry

 ** Presenting Author*