FACILE SEMI-AUTOMATED BODY FLUID IDENTIFICATION BY MULITPLEX SOLUTION HYBRIDIZATION OF NANOSTRING® BARCODE PROBES TO SPECIFIC mRNA TARGETS

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A DNA profile from the perpetrator does not reveal, *per se*, the circumstances by which it got transferred. Body fluid identification by mRNA profiling may provide contextual 'activity level' information regarding some behavioral activity on behalf of the individual that results in its transfer from the body. Here we describe the development of a prototype multiplex digital gene expression (DGE) method for forensic body fluid/tissue identification based upon solution hybridization of color coded Nanostring® probes to 23 tissue/body fluid specific mRNA targets present in forensic type samples. The body fluids/tissues targeted include peripheral blood, semen, saliva, vaginal secretions, menstrual blood and skin. To facilitate routine use, we also devised a simple 5 minute room temperature cellular lysis protocol as an alternative to standard RNA isolation for forensic sample processing using the Nanostring® procedure.

We first describe a model for gene expression in a sample from a single body fluid and then extend that model to mixtures of body fluids. From there we describe calculation of maximum likelihood estimates (MLEs) of body fluid quantities in a sample, and we describe the use of likelihood ratios to test for the presence of each body fluid in a sample. Known single source blood, semen, vaginal secretions, menstrual blood and skin samples all demonstrated the expected tissue specific gene expression for at least two of the chosen biomarkers. Saliva samples were more problematic in that their differential expression was less pronounced than with the other tissue types. Nonetheless the most specific saliva biomarker, HTN3, was expressed at a higher level in saliva than with any of the other tissues. As a preliminary indication of the ability of the method to discern admixtures of body fluids, four binary mixtures were prepared. Three of the 4 mixtures were called perfectly using the assay algorithm with no false positive results, and one of the component fluids was identified in the one 'false negative' mixture. Further optimization of the biomarker 'Codeset' will be required before it can be used in casework particularly with respect to increasing the signal to noise ratio of the saliva biomarkers. With suitable modifications, this simplified protocol with minimal hands on requirement should facilitate routine use of mRNA profiling in casework laboratories.