B78 Confirmatory Identification and Genotyping of Human Seminal Fluid Collected on Surface-Enhanced Raman Scattering (SERS) -Active Forensic Evidence Swabs

Brittania J. Bintz, MSc, Western Carolina University, 231 Natural Sciences Bldg, 111 Memorial Drive, Cullowhee, NC 28723; Katarina G. Ruehl, WCU, 90 Merlite Court, Cullowhee, NC 28723; Geraldine Monjardez, PhD, Western Carolina University, 327 Natural Sciences Bldg, 111 Memorial Drive, Cullowhee, NC 28723; and David D. Evanoff, Jr., PhD*, Western Carolina University, 231 Natural Sciences Bldg, 111 Memorial Drive, Cullowhee, NC 28723*

After attending this presentation, attendees will better understand a novel method for serological screening of forensic evidence for confirmatory identification of human seminal fluid.

This presentation will impact the forensic science community by providing results for a method that enables rapid, highly sensitive, non-destructive identification of semen. These data will ultimately be used to expand the utility of the method to other human biological fluids.

Forensic evidence is often screened to determine whether biological fluids are present prior to attempting DNA analysis. Fluid-specific serological tests employing immunological and biochemical indicators are typically used to make these determinations. Initially, a presumptive test establishes the possibility that a particular fluid is present. Next, a confirmatory test is used to identify the material and species of origin; however, these methodologies lack sensitivity and specificity. Additionally, because these tests are performed sequentially, biological fluid identification can be expensive, labor intensive, and require consumption of precious samples.

Raman spectroscopy is an optical technique that characterizes the inelastic scattering of light that is indicative of the composition of a particular molecular species. Due to the low probability of the Raman scattering event, Raman analysis of small amounts or concentrations of analyte can be problematic. SERS is an extension of the Raman spectroscopic technique in which analyte signals can be enhanced by several orders of magnitude when on or near a nanostructured metallic surface. Interaction of the electronic structures of the analyte and SERS substrate increases the analyte's Raman cross-section (i.e., the likelihood of inelastic scattering) through a chemical and/or electromagnetic enhancement mechanism. Some studies have shown that Raman spectroscopy is well suited for the analysis of biological fluids because it is rapid, highly selective, and non-destructive.[1] In the forensic laboratory, Raman spectroscopic analysis could enable a reduction in the number of serological tests performed on an evidentiary item since it can be used for simultaneous identification of all relevant biological fluids; however, Raman spectroscopy alone may not provide the level of sensitivity required for forensic samples, especially in cases in which low laser excitation powers are preferred and/or fast analysis times are required. As a result, SERS may be a more appropriate approach. This presentation describes a novel method to identify human seminal fluid on nylon-flocked swabs coated with silver nanoparticles.

For this study, sample collection swabs were prepared by synthesizing silver nanoparticles on the surface of COPAN® 4N6FLOQSwabs™ using the hydrogen reduction method.[2] Distribution and size of nanoparticles were characterized using a scanning electron microscope. Efficacy of SERS enhancement was tested by swabbing a dried sample of $[Ru(bpy)_3]^{+2}$ with a prepared collection swab, followed by detection with a Horiba LabRam HR Raman microscope. A 3X serial dilution of sole-source human seminal fluid was performed to create a total of seven samples with concentrations ranging from 15ng/µL to 21pg/µL of genomic DNA. Diluted semen (10µL) was pipetted onto the surface of swabs with and without SERS nanoparticles. Raman data of analyte adhered to the SERS-active swabs were collected using 632.8nm laser excitation and various collection geometries and integration parameters and analyzed to determine a limit of detection of the method. Following spectroscopic analysis, DNA was extracted from each swab using the Applied Biosystems® PrepFiler® Forensic DNA Extraction Kit. Several controls were also integrated during extraction. Extracts were quantified using the Applied Biosystems® Quantifiler® Trio kit. Quantifiable DNA was detected in all extracts except those obtained from swabs with low initial concentrations. Recovery was not affected by SERS particles or exposure to the Raman laser, as no difference was observed in DNA recovered from semen on naked swabs, SERS swabs, and positive controls. Additionally, quantification values were compared to Raman results to determine whether a correlation exists between SERS signal intensity and DNA recovery. DNA from a subset of samples was amplified using the Applied Biosystems® Quantifiler® kit. Full STR profiles were obtained for all samples with high starting concentrations. As expected, stochastic effects were observed in data obtained from samples with the lowest starting concentrations; however, no differences were observed in DNA profiles from SERS extracts versus non-SERS extracts. These results demonstrate that confirmatory identification of human seminal fluid using SERS is robust, sensitive, and does not affect downstream analyses.

References(s):

1. Kelly Virkler and Igor V. Lednev. Raman spectroscopy offers great potential for the nondestructive confirmatory identification of body fluids. *Forensic Sci Int*. 181 (2008): e1-e5.

2. David D. Evanoff and George Chumanov. Size-controlled synthesis of nanoparticles. 1. "Silver-only" Aqueous Suspensions via Hydrogen Reduction. *J Phys Chem B*. 37 (2004): 13948-13956.

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