INTERNAL VALIDATION OF SPERM HY-LITER™ FOR THE IDENTIFICATION OF SPERM CELLS

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The Nuclear DNA Unit (nDNAU) at the Federal Bureau of Investigation Laboratory has recently validated an immunofluorescence differential staining technique known as SPERM HY-LITER™ for the identification of sperm cells on evidentiary type items as supplemental means to traditional methods of sperm cell identification.

SPERM HY-LITER™ utilizes a multi-chemical approach to affix material, enhance fluorophore binding and block non-specific fluorophore-antibody interactions. The sperm cell staining reagents exploit a fluorescently labeled, monoclonal anti-sperm head antibody that binds specifically to sperm head antigen. Latent binding of an existing Alexa Fluor® 488 fluorophore-conjugated antibody-antigen complex is visualized via fluorophore excitation and photon emission using fluorescence microscopy coupled with fluorophore-specific filtration cubes. SPERM HY-LITER™ also includes a second, non-specific DAPI fluorophore that intercalates within nucleic acids and provides identification of cell nuclei.

The nDNAU previously initiated internal validation studies using single-source and mixed-source slide preparations consisting of semen, vaginal, buccal, blood and urine specimen types to methodically assess the suitability and reliability of SPERM HY-LITER™ for casework specimens. The primary purpose of these studies was to evaluate the sensitivity, specificity, cross-contamination susceptibility, and DNA recovery capability of specimens subjected to SPERM HY-LITER™ processing. Additional studies were conducted to assess fluorophore stability over time and downstream STR typing capability. Supplemental studies were recently initiated to further assess the sensitivity and specificity of additional specimen types including menstrual blood, vaginal yeast and rectal swabs. The photostability of Alexa Fluor® 488 and DAPI fluorophores were also assessed.

SPERM HY-LITER™ validation results demonstrated detection sensitivity to a single sperm cell with the enhanced investigative ability to identify sperm cells undetected under phase contrast microscopy alone. Results further demonstrated no fluorophore cross-label/reactivity amid various specimen types and no apparent inhibition of downstream DNA extraction, PCR amplification or capillary electrophoresis procedures. SPERM HY-LITER™ can increase evidence throughput by reducing overall evidence slide examination times using lower magnification scanning requirements. Limitations observed included specimen susceptibility to non-specific fluorophore staining, varying levels of background fluorescence, rapid DAPI fluorophore photobleaching, longer staining requirements, and cost of reagents and equipment.