INTERNAL VALIDATION OF THE SPERM HY-LITER™ KIT FOR THE IDENTIFICATION OF HUMAN SPERM CELLS IN FORENSIC SAMPLES

Jordan M. Anderson, B.S.; Brian R. Fischer, B.S.; Kevin W.P. Miller, Ph.D.
Human Identification Laboratory, California State University of Fresno, Fresno, CA

The Sperm HY-LITER™ kit, developed by Independent Forensics (IF), employs a novel antibody technique to positively identify human sperm heads from forensic stains. More conventional microscopic techniques use slide stains that are often non-specific, requiring time-consuming examination by the analyst and resulting in a greater chance of false negative results. By using an Alexa 488 fluorescent dye-labeled mouse monoclonal antibody system that specifically binds to the heads of human sperm, the HY-LITER™ kit boasts a degree of specificity and sensitivity that is unmatched by current sperm visualization techniques. Once the dye is applied, spermatozoa are easily seen using a fluorescent microscope with a fluorescein isothiocyanate (FITC) appropriate filter. Moreover, the kit also uses 4, 6 diamidino-2-phenylindole (DAPI), a second, non-specific fluorescent stain, to simultaneously bind to the DNA of all cell nuclei within the sample. The DAPI-stained cells can be visualized just as easily using a fluorescent microscope with the appropriate DAPI filter.

Although monoclonal antibody technology is used in manufacture of the Sperm HY-LITER™ kit, and the semen of a variety of species were tested during developmental validation, the developmental work failed to directly demonstrate the Sperm HY-LITER™ dye is specific only to human sperm cells. Non-human primate sperm samples were not tested for possible interference or false positives. Therefore, this internal validation study was designed to specifically address that shortfall. The sensitivity, specificity, and reproducibility of the HY-LITER™ kit were tested by performing multiple slide comparisons under solitary and mixed sample conditions to identify potential sources of false positives and reaction inhibitors. Tests were conducted using samples of human semen, saliva, blood, and urine, non-human primate (Macaca mulatta and Macaca fascicularis) semen, and dog (Canis lupus hallstromi) semen.

Our most significant finding was the reproducible absence of a positive fluorescent signal when applying the kit dyes to non-human primate sperm samples. The results of our studies continue to support the manufacturer’s claim that the Sperm HY-LITER™ kit is specific only to human sperm heads. Additionally, our results consistently reproduced the results of the manufacturer’s developmental validation using samples from other mammals. The incorporation of the Sperm HY-LITER™ kit into the standard operating procedures of forensic laboratories should greatly increase the throughput of case samples while simultaneously improving the sensitivity, and specificity serological screening procedures. It will also improve the analyst’s ability to attribute a source to an unknown forensic stain. The Sperm HY-LITER™ kit also has the potential to eliminate sperm visualization problems involving microscopic laser dissection, furthering the development of automated platforms for the retrieval and molecular genetic analysis of human sperm cells.