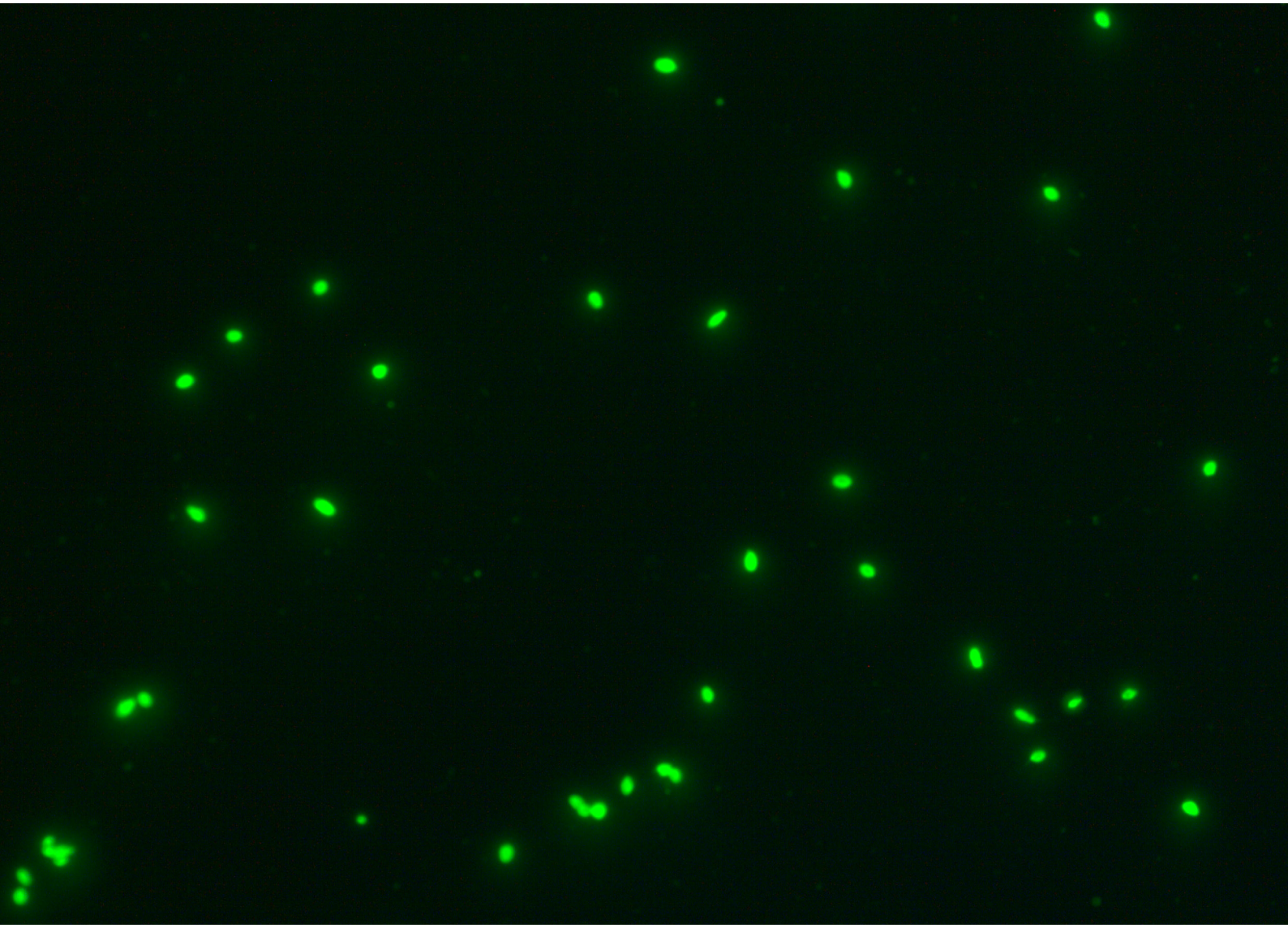
Application Note



Microscopic Detection of Sperm on Washed Textiles After HY-LITER Staining

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Microscopic Detection of Sperm on Washed Textiles After HY-LITER Staining

Authors: Galina Kulstein
Institute of Forensic Medicine at Ulm University, Germany

Dr. Ulrike Schacker, Martin Schatzl
Galantos Genetics Laboratory, Mainz, Germany

Thorsten Kern, Dr. Michael Gögler
Carl Zeiss Microscopy GmbH, Germany

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The forensic testing of DNA samples is an important part of day-to-day forensic activities. Often, weeks or months pass between the committing of a crime and the analysis of the evidence by forensic geneticists, and during which time evidence relevant to the crime is washed. This study shows that even after two washing cycles at a water temperature of 60 °C, a sufficient number of sperm cells can still be detected to be used to create a genetic fingerprint.

Introduction

The ability to identify spermatozoa is a particularly important technique in forensic science, for example within the scope of solving sexual crimes. A suspected perpetrator's sperm needs to be detected on the victim's clothing and identified through the use of a specific test. This is achieved, for example, through the use of RSID-Semen test kits, which react positively to semenogelin (a protein from the male seminal vesicle). With the help of short tandem repeat (STR) typing, otherwise known as the "genetic fingerprint," it is possible to match a specific individual profile to the person who left the sample.

Rapes and sexual assaults are not always reported right away. Often, weeks or months pass between the committing of a crime and the analysis of the sample by forensic geneticists. During this period of time, evidence relevant to the crime, like the victim's bedclothes or clothing, is frequently washed once or multiple times. Systematic analyses conducted as part of several studies have already shown that it is possible to extract DNA and create an STR profile from evidence that has been washed [1, 2]. It was unclear, however, whether it was also possible to microscopically identify spermatozoa using antibody-based fluorescence detection (SPERM HY-LITER) and identify semenogelin (RSID-Semen test) after the evidence was washed once or twice in a washing machine.

Structure and Function of Human Spermatozoa

Spermatozoa are flagellated cells that occur in the ejaculate of male individuals and serve to fertilize female reproductive cells. Morphologically, they can be divided into three characteristic sections (fig. 1). The sperm head with the nucleus, which is the carrier of the haploid set of chromosomes, is located on the front side. On top of the front side is the acrosome, a cap-like structure filled with enzymes that help the sperm penetrate the egg cell. The middle piece, the neck, contains a large number of mitochondria that provide energy for the sperm's motility. The flagellum forms the end of the spermatozoon. This axial filament system of microtubules is also for motility.

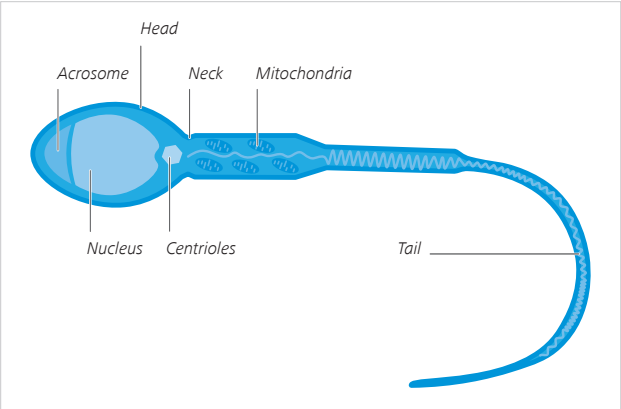

Figure 1 *Structure of a spermatozoon*

Experimental Design

Before machine washing, 100 µl, 20 µl, and a dilution of 1:10 µl of unaltered semen was applied to pieces of fabric consisting of 100 % cotton and left to dry overnight at room temperature. Afterward, the items of clothing to which the semen samples were applied were washed in a washing machine (Miele Softronic W 3741) using powdered laundry detergent (Ariel Color). A wash program was selected with a duration of 140 minutes and a spin cycle with a speed of 1,000 rotations per minute (rpm). Spermatozoa identification was carried out after one wash cycle and after a second wash cycle under the same conditions. For the positive control (fig. 4), a piece of cotton was extracted with 20 µl of semen applied.

RSID-Semen Test and SPERM HY-LITER Detection

The semen stains marked with a waterproof fabric marker were cut out of the washed pieces of fabric and each was extracted in 700 µl RSID-Universal Buffer. For the RSID test (an antibody-based thin-layer chromatography test), 100 µl of the extract was pipetted onto the test cassette and the result was read after 10 minutes (fig. 2).

The piece of fabric was centrifuged in a DNA IQ™ Spin Basket (Promega Corporation) for three minutes at 10,000 rotations per minute (rpm). This extract was then added to the first extract and centrifuged once again. Any additional extract above approx. 60 µl was discarded. Of the retained amount, 2–10 µl was applied to a specimen slide and used for the HY-LITER staining. This was carried out according to the manufacturer's instructions.

HY-LITER staining is based on a fluorescent-labeled antibody that binds to the sperm head. At the same time, the nucleus is also stained with 4',6-diamidino-2-phenylindole (DAPI). When using the Alexa 488 filter, the sperm exhibit green fluorescence under the microscope (fig. 3, 4). When using the DAPI filter, the nucleus of all cells, i.e. epithelial cells and sperm cells, appears blue. Using this staining method, sperm cells can be clearly differentiated from all other cells. The rest of the sperm solution was used for the DNA extraction process. Isolating the sperm's DNA for the STR analysis was carried out using the QIAamp DNA Investigator Kit (QIAGEN).

Recommended Microscope Equipment

An Axio Scope.A1 light microscope (fig. 5) was used together with an HXP 120 illuminator and the Axiocam ERc 5s microscope camera. Documentation of images was carried out using the ZEN imaging software and an iPad. This was connected to the microscope camera via WiFi. Image capturing and processing was easy and possible without any prior experience with the software.

Procedure

The samples were prepared and processed simultaneously at the laboratory for forensic science in Ulm and at Galantos Genetics' laboratory in Mainz.

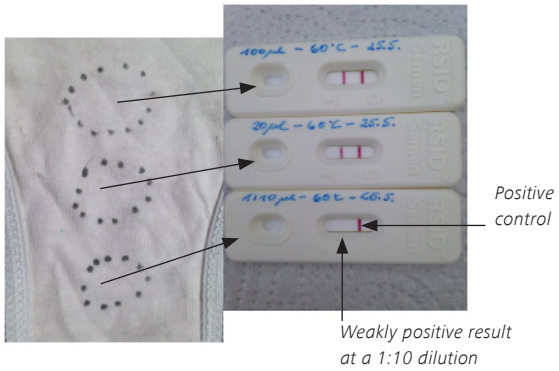

Figure 2 *RSID detection of samples: 100 µl, 20 µl, and a 1:10 dilution were applied to the piece of fabric. All of the samples were washed once at 40 °C or once/twice at 60 °C.*

The results show that immunological sperm detection by means of an RSID test is successful both with weaker (sample volume of 20 µl) as well as more concentrated applications (sample volume of 100 µl) that were washed using laundry detergent at either 40 °C or 60 °C. Furthermore, it was still possible to detect semenogelin on the fabric after being washed twice at 60 °C, even when the sample was applied as a 1:10 dilution. Furthermore, regardless of the volume of the sample applied and the washing temperature, sperm was detected using antibody-based fluorescence detection by means of a SPERM HY-LITER test. As was expected, the number of detected sperm cells both declines as the washing temperature increases and as the volume of semen applied declines, as well as in the event of multiple washes.

Sample no.	Sample	Washing temperature	µl applied	RSID	HY-LITER	DNA perpetrator profile creation
1	Cotton	40 °C	100	Positive	Approx. 1,000 spermatozoa	Positive
2	Cotton	40 °C	20	Positive	Approx. 800 spermatozoa	Positive
3	Cotton	60 °C	100	Positive	Approx. 380 spermatozoa	Positive
4	Cotton	60 °C	20	Positive	70 spermatozoa	Partially positive
5	Cotton	60 °C	1:10	Weakly positive	None in 10 µl extract	Partially positive
6	Cotton	2×60 °C	20	Weakly positive	47 spermatozoa	Partially positive

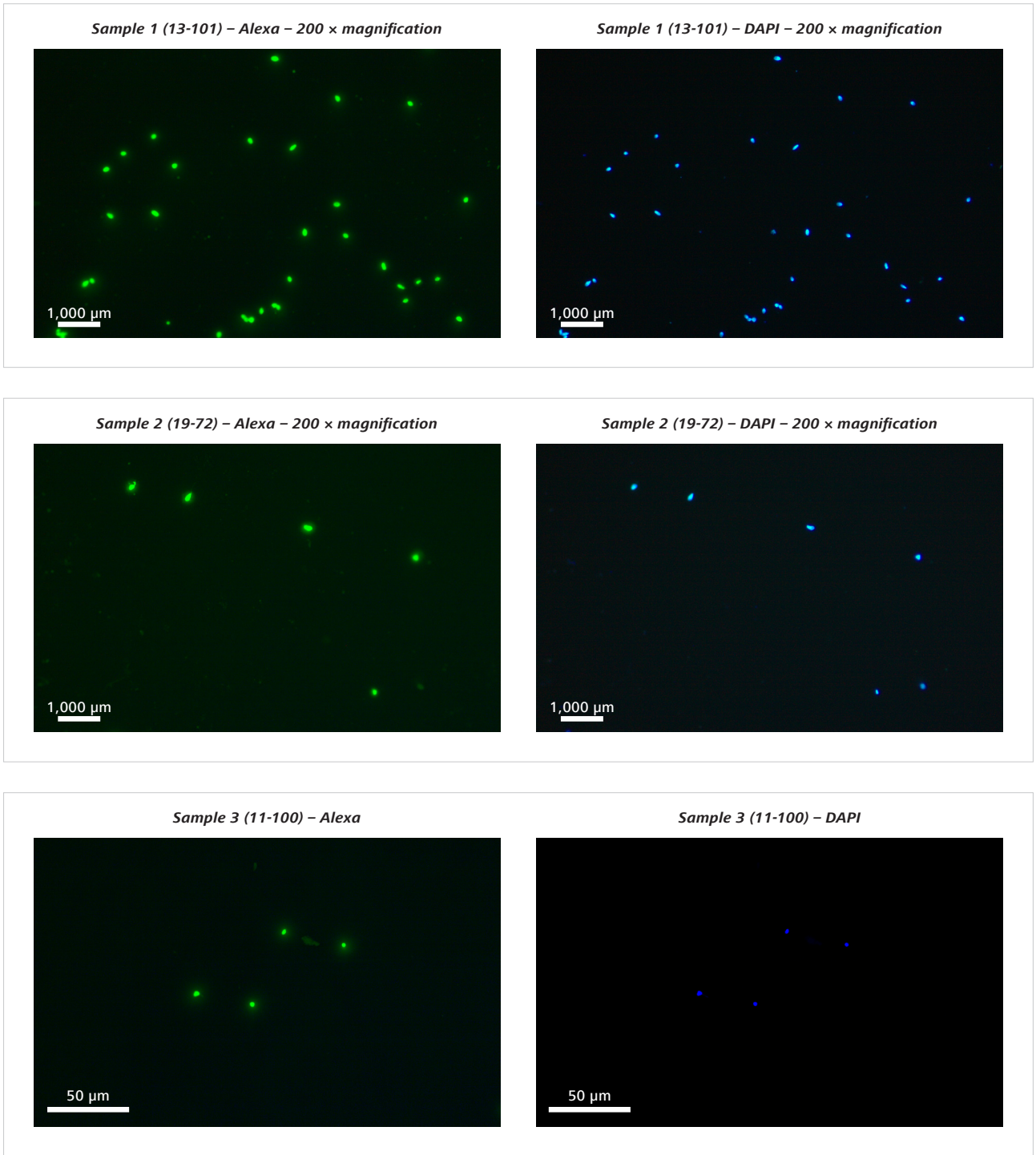

Figure 3 *Individual samples: Antibody-based spermatozoa staining with HY-LITER (green fluorescence) and simultaneous nucleus staining with DAPI*

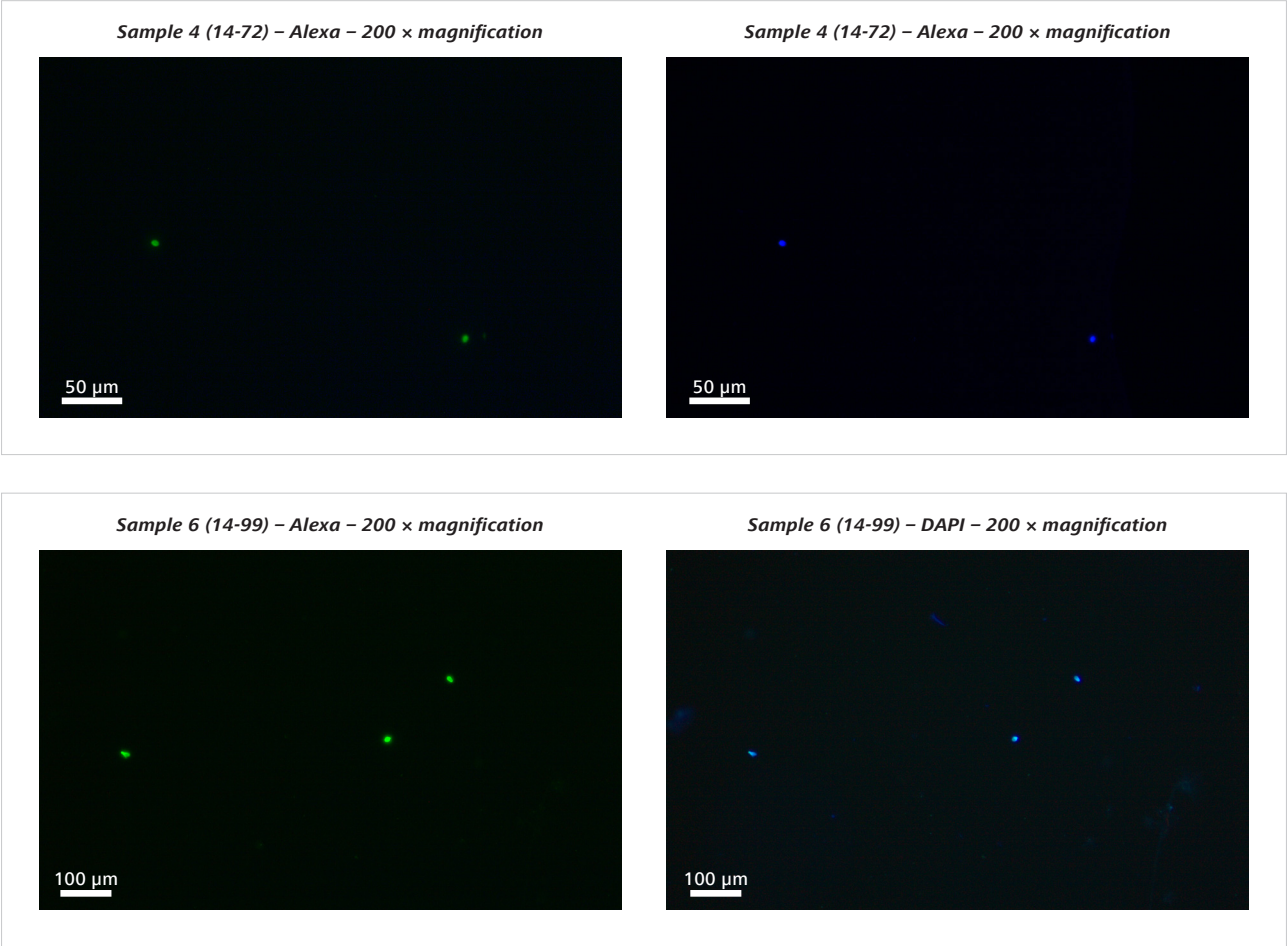

Figure 3 *Continuation*

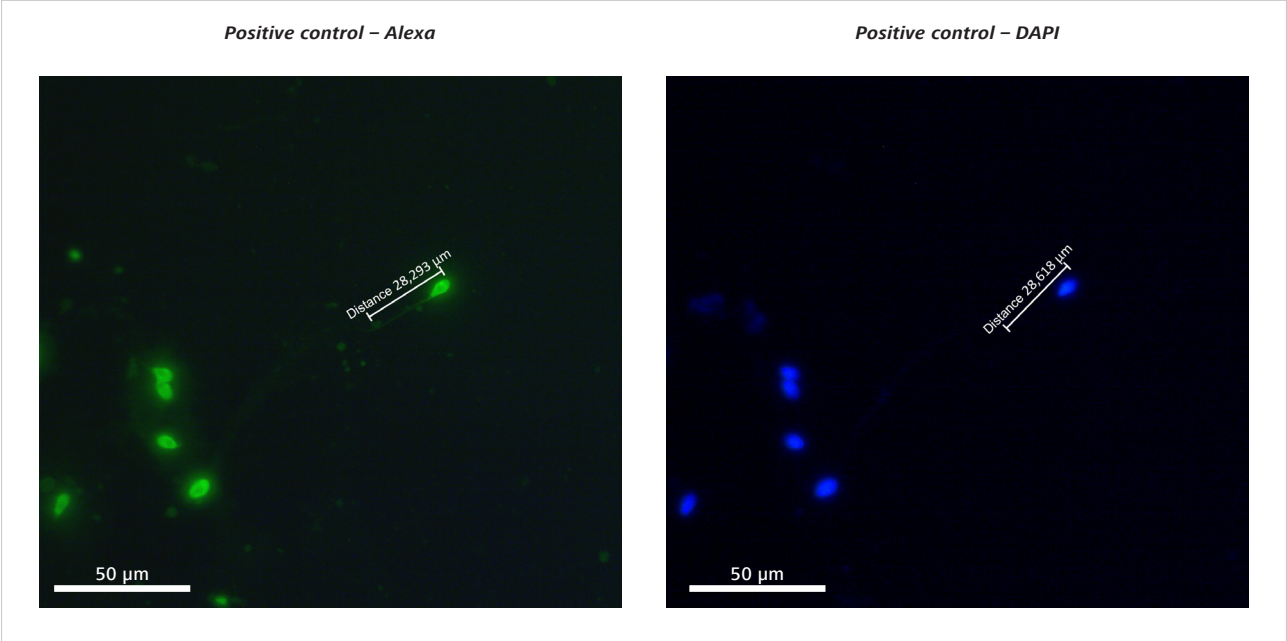

Figure 4 *Positive control – HY-LITER: Antibody-based spermatozoa staining with HY-LITER (green fluorescence – Alexa 488) and simultaneous nucleus staining (blue fluorescence – DAPI)*

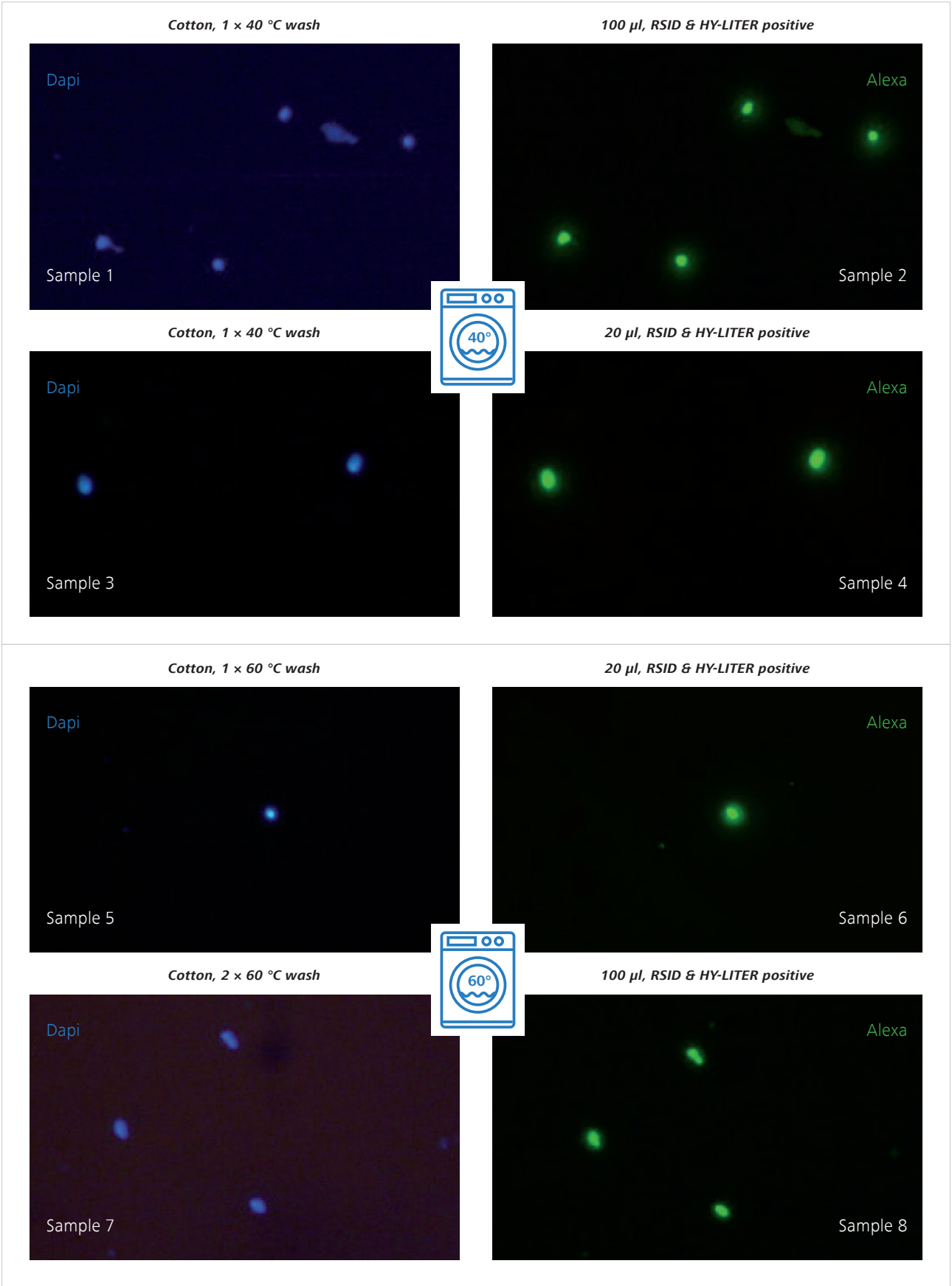

Figure 5 *Spermatozoa identification on washed textiles using HY-LITER staining*

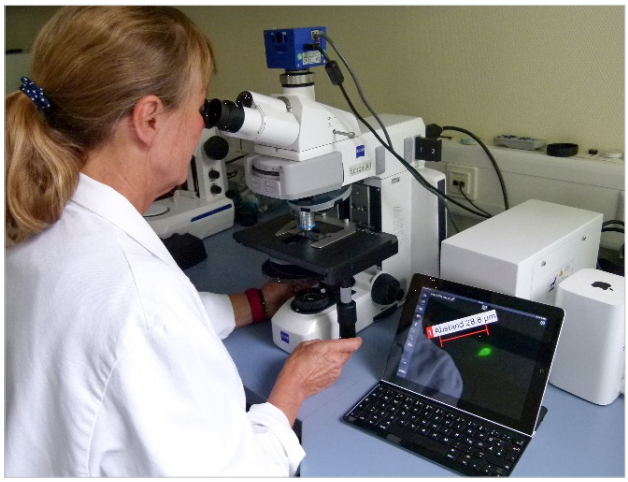

Figure 6 *ZEISS Axio Scope.A1 fluorescence microscope*

Experiments have already shown that approximately 60 pg of DNA can be extracted from 20 sperm cells, and that this quantity is sufficient to carry out STR typing [3]. As such, the subsequent DNA testing of all the analyzed samples produced either positive or partially positive results, and made it possible to successfully identify the individual who left the sample.

Conclusion for Forensic Casework

This study shows that it is worthwhile to test washed articles of clothing for the purpose of investigating a sexual crime, since a sufficient number of sperm cells can still be detected even after two washing cycles at a water temperature of 60 °C. This means that the DNA is still intact and, as a result, can be used to create an STR profile, a genetic fingerprint of the perpetrator.

References:

[1] Andrews C, Coquoz R (1994) PCR DNA typing of washed stains, In: Walter Bär, Angelo Fiori, Umberto Rossi (ed.) Advances in Forensic Haemogenetics 5, Springer Verlag, Heidelberg, pp 343–345.

[2] Brayley-Morris H, Sorrell A, Revoir AP, Meakin GE, Courts DS, Morgan RM (2015) Persistence of DNA from laundered semen stains: implications for child sex trafficking cases. Forensic Sci Int Genet. 19:165–171.

[3] Schneider C, Müller U, Kilper R, Sibertz B (2012) Low copy number DNA profiling from isolated sperm using the aureka®-micromanipulation system. Forensic Sci Int Genet. 6:461–465.

Carl Zeiss Microscopy GmbH
07745 Jena, Germany
microscopy@zeiss.com
www.zeiss.com/axioscope