



Comparative study between a new forensic reagent STK™ SPERM TRACKER versus multispectral Alternative Light Sources (ALS) to detect specifically sperm traces on swab and fabrics in real sexual assault cases.

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Background :

It is estimated that more than 5 million rapes are committed globally each year. For 1 000 rapes, only 4.6 rapists will go to jail because rape is highly under-reported but also because classic **alternative light sources (ALS)** miss a great number of **semen stains**. Specific semen stains localization is an important challenge amongst the variety of biological fluids present on evidences. These detections are often performed using non-fully specific techniques, leading to a great proportion of false positives and even worse, false negatives (missed stained).

A product from **STK™ Sperm Tracker** portfolio has been developed in order to detect and locate specifically semen traces on fabrics, by emitting a fluorescent blue signal : STK™ Lab (pre-impregnated and ready-to-use Paper) with the combination of a 365 nm UV lamp. The manufacturer does not recommend the use of STK™ Lab for swabs, however it was evaluated in this study.

Objective :

The objective of the study is the:

- Comparison between classical lamps examination and the newly available product STK Lab™
- Determination of STK Lab™'s specificity vs spermatozooids observation / male profile determination

Materials & Methods :

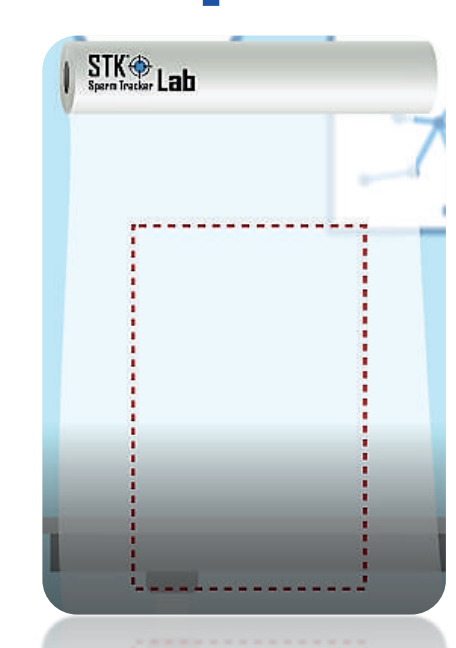
Spermatozooids observation: 1 cm² of fabric containing the stain or the swab head are incubated 5 minutes at 37°C with trypsin solution (0.25%) and centrifuged 3 minutes at 14000 rpm using spin baskets (DNA IQ Spin baskets, PROMEGA). The pellet is resuspended in 50µl of PBS 1X. 10µl are spotted on microscope slide and dried in a steamroom (at least 10 minutes at 56°C). Spermatozooids are tagged using Nuclear Fast Red (NFR) coloration. Spermatozooids are detected and counted using the Panoramic MIDI 2 BF scanner (designed and manufactured by 3DHISTEC) at 20x zoom scan and by using the detection software Calopix (by TRIBVN HEALTHCARE).

Male profile determination: Differential DNA extraction is performed from the pellet resuspended using Ademtech chemistry kit E-Lysis Buffer kit and Crime Prep Adem-kit, in order to separate male fraction and female fraction. DNA profile are obtained after DNA quantification (Quantiplex Pro, Qiagen), PCR amplification (GlobalFiler IQC, ThermoFisher) and CE (3500XL, ThermoFisher (Injection voltage=1.2 kV, injection time = 24 sec).

ALS obsrevation was performed under a CrimeLite2 UV illumination, with yellow filtered goggles, and Crime Lite ML PRO UV spot.

STK™ Lab was purchased from AXO Science (FRANCE). Ref. AXO-STK-9240. Observation was performed using a UV lamp Vilber VL6L (365 nm).

STK™ Lab protocol :



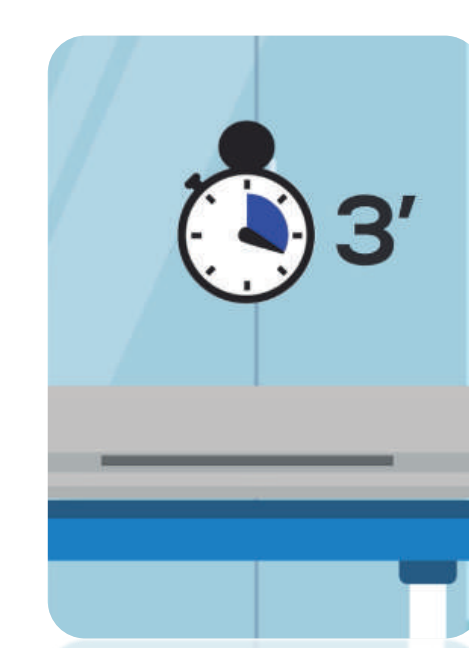
Cut a piece of STK Lab the size you need to cover the evidence



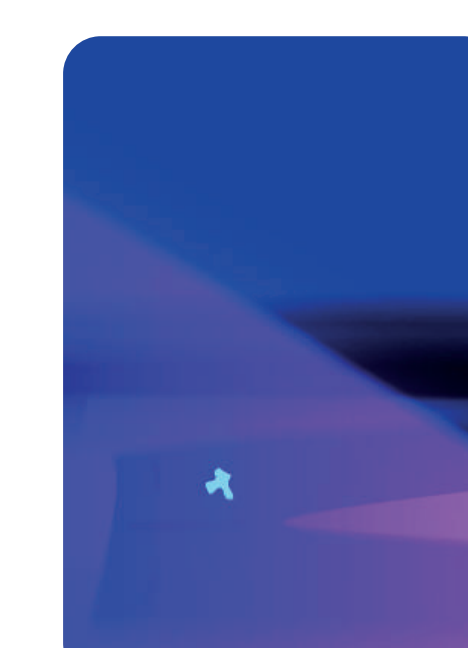
Spray impregnated size of STK Lab paper with **water** (must be saturated)



Put wet absorbing side against the evidence (you can pin or staple)



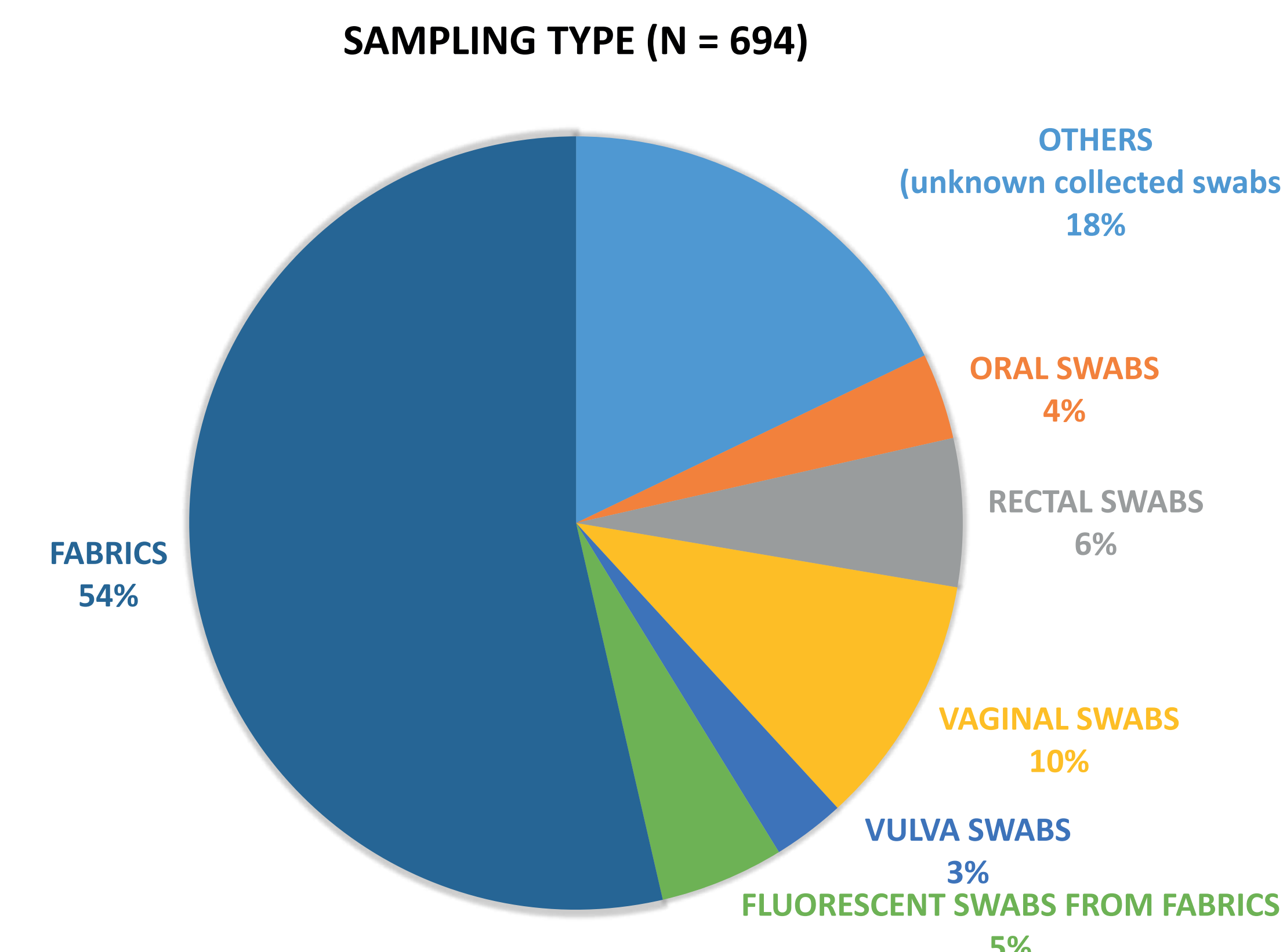
Press 3 minutes



Semen traces appear clearly under a 365nm UV lamp

Study :

Comparison between classical forensic lamps examination and the newly available product STK Lab™ + the 365 nm UV lamp on casework samples (fabrics and intimate swabs).



Example of the different observations made on fabrics, here on a sock



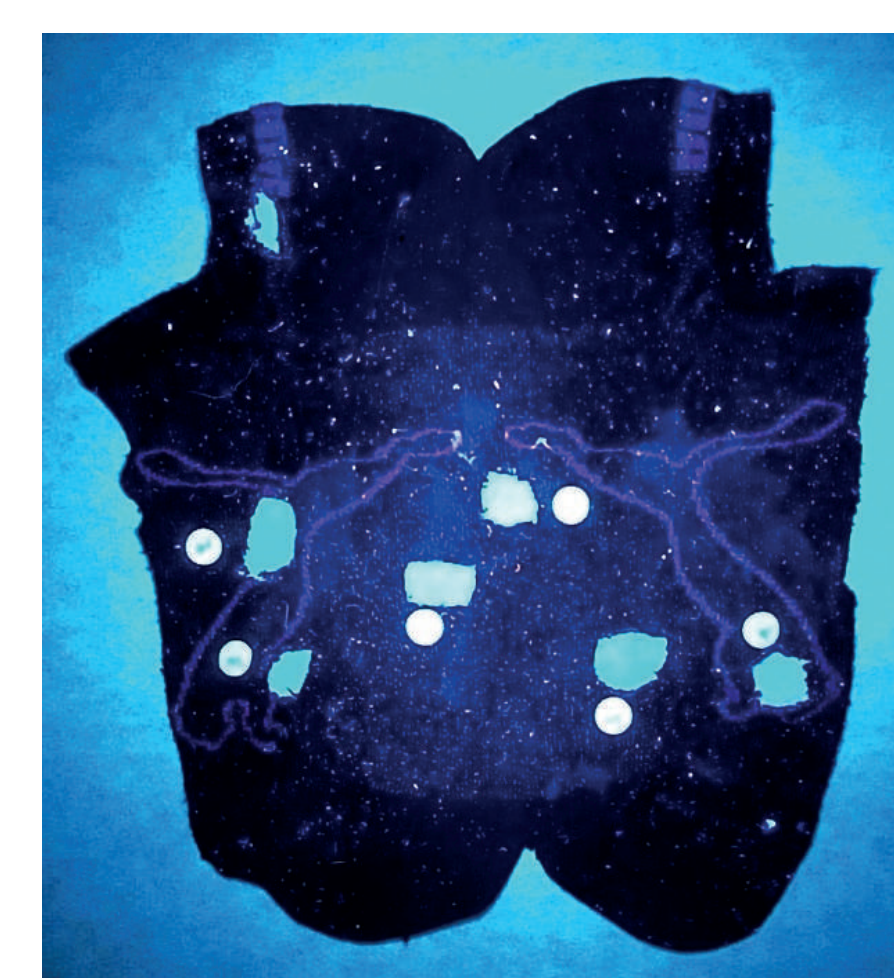
Visible Light



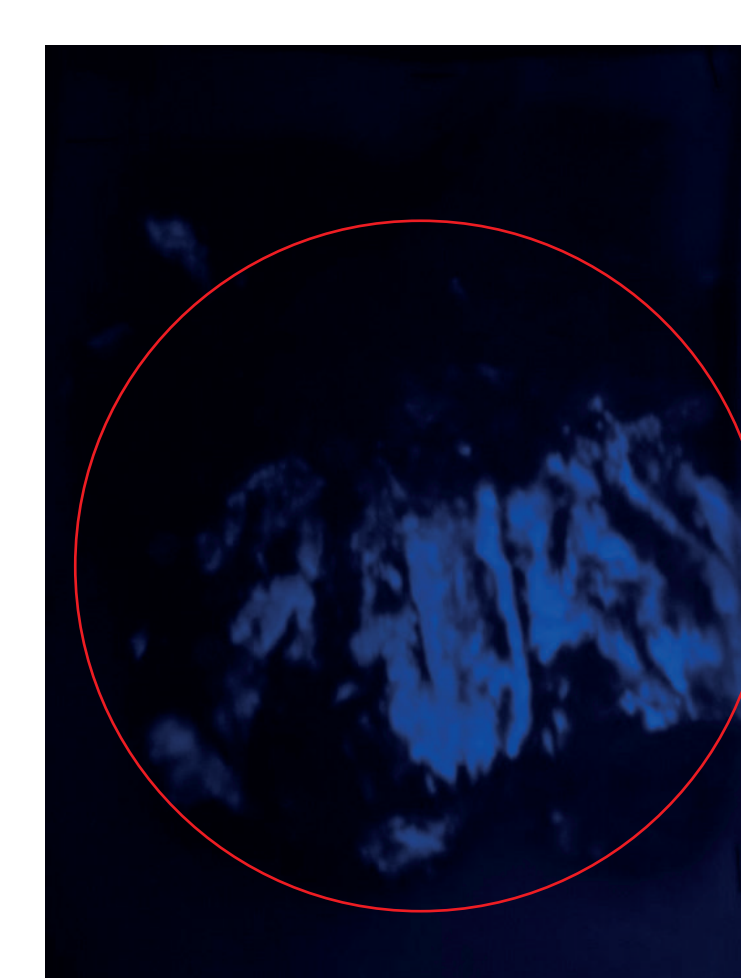
Crime Lite Blue



Crime Lite Violet

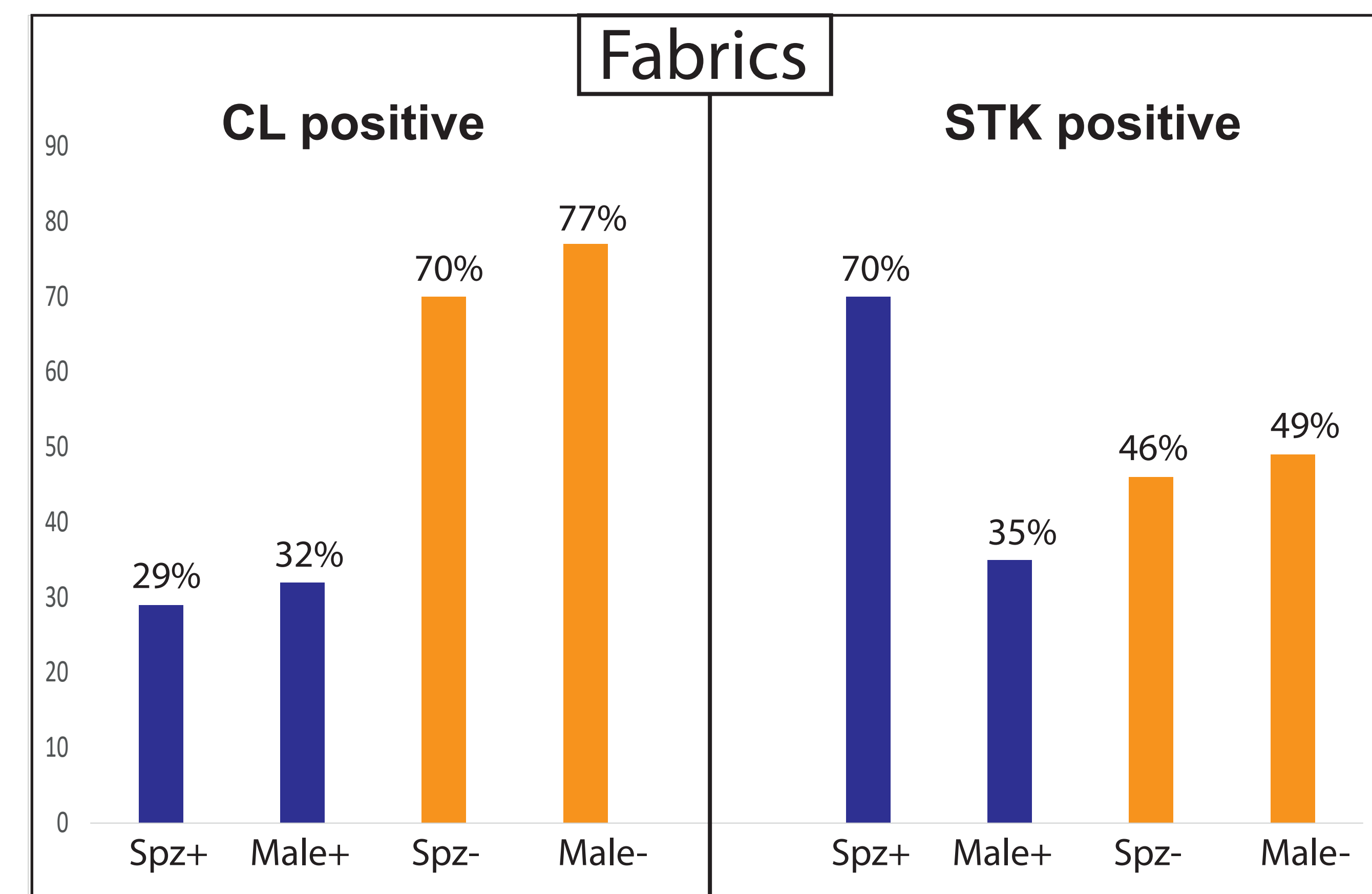


ML PRO UV Spot-LP420



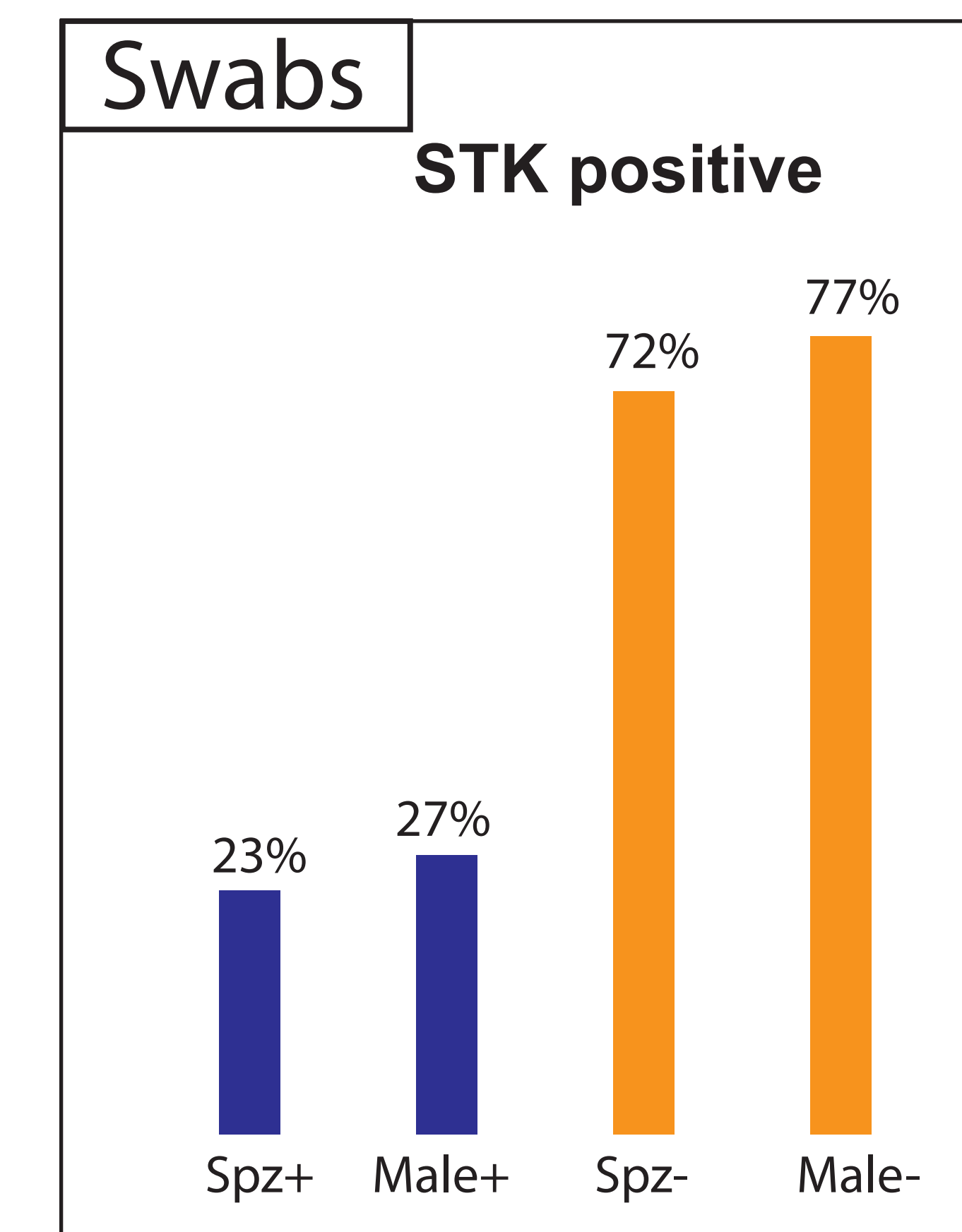
STK Lab UV 365nm

- No stain is visible or detectable by using regular lamps examination with blue or violet filters.
- A large but vague fluorescent area is noted with ML PRO UV SPOT LP 420 lamp.
- **STK Lab™** (applied on the surface of the sock) reveals specifically sperm stains within the fluorescent area. These observations with STK Lab™ lead to positive spermatozoid research and **human male genetic profile**.



CL = Crimelite
STK = Sperm Tracker
Spz = Spermatozoid presence
Male = Male profile

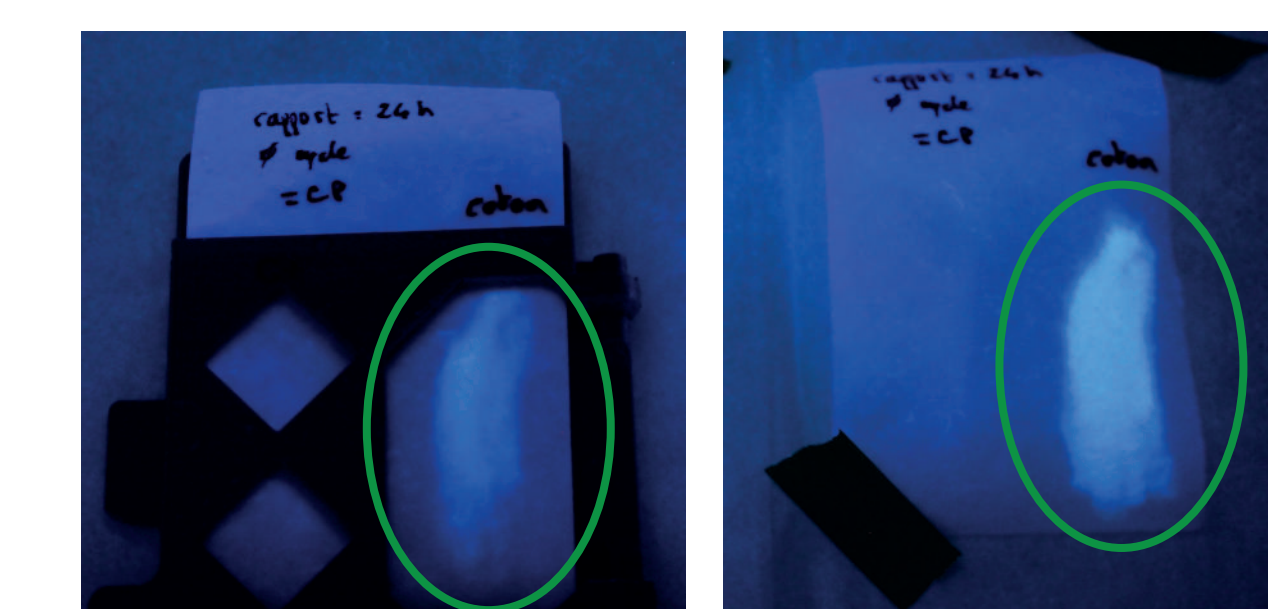
STK improves the performances on fabrics both by reducing the ratio of false positive (Spz -, male -) and by increasing the specificity (Spz +, male +).



STK test can be performed on swabs, whereas ALS cannot be used (coton and fibers autofluorescence). Intimate swabs give a high false positive ratio (spz -, male -) resulting by a blue signal at low intensity due to phosphatase acid at low concentration in vaginal secretions.

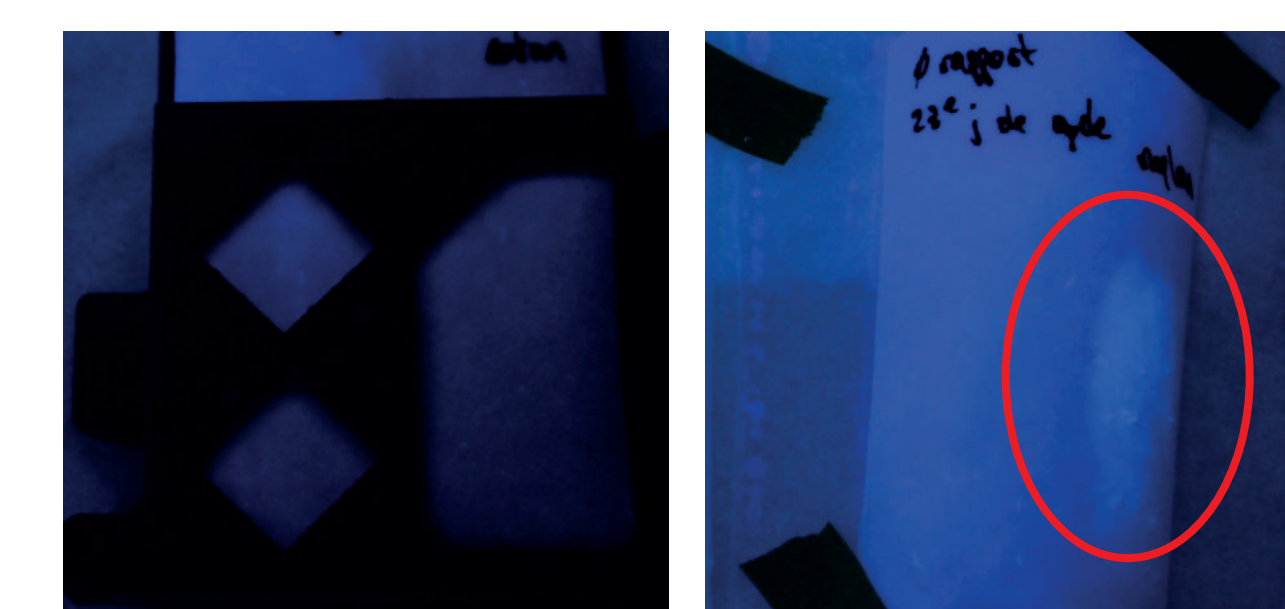
Specificity optimization from vaginal swabs :

Swabbing 24 hours after sexual intercourse with ejaculation (mix of sperm and vaginal fluids)



Positive signal (regular) 20 sec post trasnsfer
Positive signal (strong) 5 min post trasnsfer

Swabbing after absence of sexual intercourse (only vaginal fluids)



No signal 20 sec post trasnsfer
No specific signal (weak) 5 min post trasnsfer

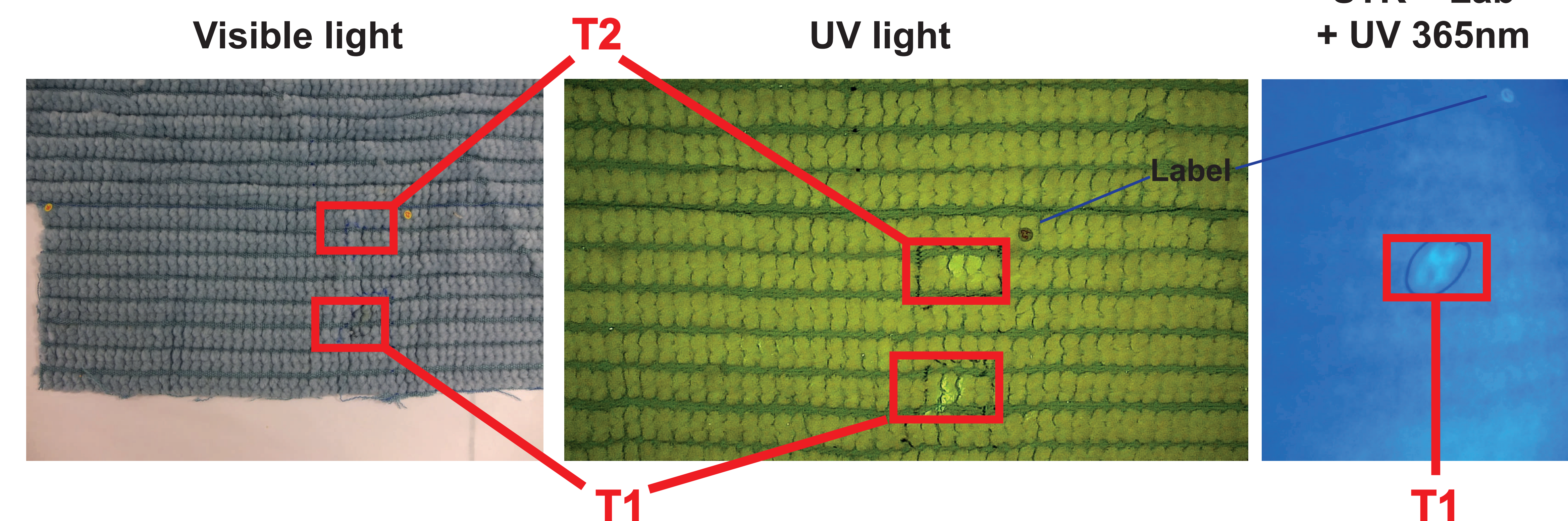
Specific signal appears quickly (20 seconds) after the reaction between STK Lab paper and prostatic acid phosphatase.

Non Specific signal appears around 5 minutes after the reaction between STK lab paper and vaginal acid phosphatase.

The transfer was performed by rolling the swab head on the STK lab.

False positive results from vaginal swabs can be reduced by just rolled the head of swab on STK Lab paper (no press needed) and read the presence/absence of signal after only 20 seconds

STK™ Lab on a 25 years old evidence, to solve a cold case



- Positive revelation with **STK™ Lab on a 25 years old case**: confirmed with spermatozoid observation and **human male genetic profile** determination. T1 shows a clear specific signal

Conclusion

STK™ Lab is an innovative strategy to detect specifically sperm traces on swab and fabrics in real sexual assault cases. By targeting the human Acid Phosphatase, STK improves the performance by reducing the false positive number, and by avoiding missing stains compare to regular lamps examination.

STK™ Lab can be used in cold case (> 25 years).