ENHANCED SEMEN ELUTION FROM 4N6FLOQSwabs™ AND COTTON SWABS PRIOR TO DNA ANALYSIS

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Introduction: Seminal fluid detection from evidences of sexual aggression is a high priority in the forensic laboratory. Investigation of seminal fluid from sexual assault victims could be problematic for genetic identification because samples, currently collected with cotton swabs, have a low sperm cell concentration. It was reported in Benschop et al. (2010), that vaginal postcoital samples collected with nylon flocked were found to maximize cell capture for genetic analysis, however, a low number of samples were studied and sperm detection variation could not be properly evaluated as there was no control sample.

Objectives: In this study we compared: 1) the quantity of seminal fluid eluted from sample collected with nylon *4N6FLOQ™Swab* (*Copan Flock Technologies, Brescia, Italy*) and cotton swabs. 2) Challenge the sperm elution capabilities of both nylon *4N6FLOQ™* and cotton swabs with different testing conditions like temperature, time, pH/detergent presence, shaking and proteinase K (ProK) digestion. 3) Quantify the amount of sperm, PSA and semenogelin (Sg) antigens captured by nylon *4N6FLOQ™Swab* and cotton swabs.

Material & Methods: 240 evidences were stained with semen from normospermic and oligospermic donors. The varying factors investigated for spermatozoa recapture were: temperature of 24ºC-42ºC, 30 min-1h time, elution volumes of water/PBS at pH 8.5 +/- SDS 1%, as well as, vortex shaking and ProK at 56ºC. Semen was evaluated for PSA with *Seratec,* and for semenogelin antigen with the *RSID™-Semen assay.* Statistical analyses of means and variances were carried out.

Results & Discussion: *4N6FLOQ™Swab* significantly enhanced the release of captured spermatozoa (*p<0.001, df=168*). A three-fold increase was obtained from nylon (74±5%) with regard to cotton swabs and such may confirm the DNA increase previously reported by Benschop. ANOVA analyses showed significance in the *swab type*time* factors interaction (*p≤0.01*) for normospermic semen, as well as with *temperature* and *time*temperature* (*p≤0.05*) for both normospermic and oligospermic elutions. Within cotton swabs, 42ºC and vórtex mechanical agitation doubled the spermatozoa counts; anionic detergent SDS elution and 56ºC-10ug/mL ProK digestion optimized captures 20% and three-five times, respectively. The 30-min incubation was enough for optimum elution reaching a plateau. On the other hand, varying volume conditions affected non-spermatic markers termolability. PBS (pH 8.5) obtained maxima detections of PSA and Sg at 1/1.200.000 and 1/100.000 dilutions, respectively, although that was not good for spermatozoa visualization due to nuclear basiphication. The Copan nylon *4N6FLOQSwab™* is hereby recommended as an optimum device for sexual-assault sampling, specially coming from presumptive scarce-sperm evidences.