Validation Studies of the Sample Collection System for Forensic Use

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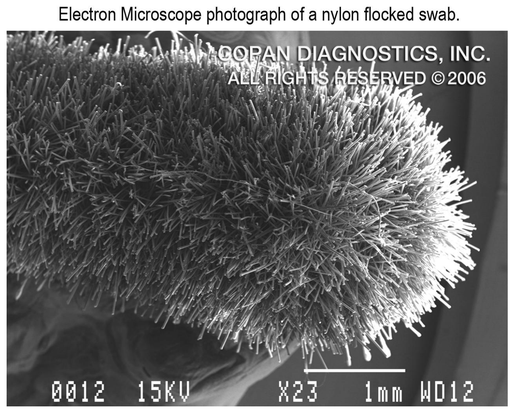
Introduction

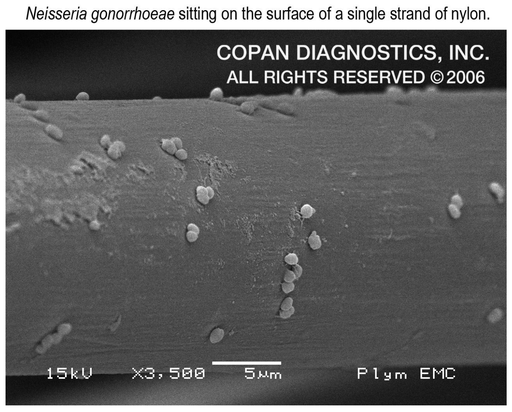
The aim of this presentation is to inform the forensic community about the results of the validation studies performed on the novel sample collection system – flocked swabs – designed specially for the forensic use. Sample collection capacity, assay sensitivity, DNase-free, RNase-free, human DNA-free, PCR-inhibitor free status, and a suitability for automated DNA extraction are the core features of the flocked swabs. 4N6 DNA flocked swabs can be used not only for reference sampling (mouth swabs) but due to its sampling capacity and especially the efficiency of sample release from the swab matrix are extremely suitable for crime scene sampling. The key factor influencing the suitability for CS sampling is the » 100% release rate of the biological material from the swab. The results of experiments proved the expected high efficiency of sample release from the flocked swabs in different extraction buffers.

Picture gallery

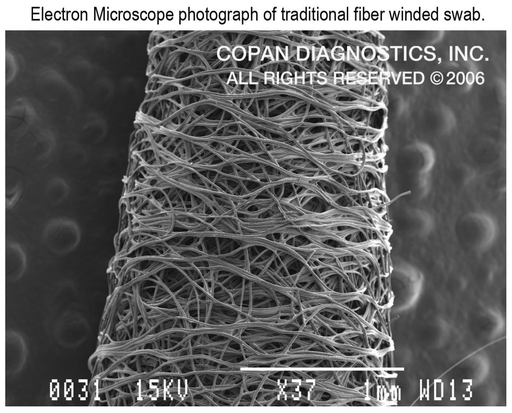
Electron Microscope photograph of a nylon flocked swab.



Neisseria gonorrhoeae sitting on the surface of a single strand of nylon.



Electron Microscope photograph of traditional fiber winded swab.



Results

Spontaneous release of cells from the 4N6 flocked swabs during 2 minutes incubation in different extraction buffers

Serie a) all swabs incubated 2 minutes in the respective lysis buffer, swabs removed (A), extraction continued with supernatant; **Serie b)** swabs from (A) placed to a new tube, new extraction buffer added, and samples processed accordingly to the manufacturer s extraction protocol (B) + centrifugation through the spin baskets.

4N6 flocked swabs – DNA IQ (Promega)

Assay	Ct SYBR	ng DNA/ µl
4N6_DNA IQ_1a	9.43	0.43
4N6_DNA IQ_1b	9.17	0.51
4N6_DNA IQ_2a	9.52	0.40
4N6_DNA IQ_2b	9.67	0.38
4N6_DNA IQ_3a	10.44	0.22
4N6_DNA IQ_3b	9.86	0.33
4N6_DNA IQ_4a	10.36	0.24
4N6_DNA IQ_4b	10.43	0.23

4N6 flocked swabs – DNA Micro kit (Qiagen)

Assay	Ct SYBR	ng DNA/ µl
4N6_Qiagen_1a	3.81	15.38
4N6_Qiagen_1b	5.01	7.16
4N6_Qiagen_2a	3.39	20.11
4N6_Qiagen_2b	4.28	11.40
4N6_Qiagen_3a	4.36	10.83
4N6_Qiagen_3b	4.53	9.72
4N6_Qiagen_4a	4.11	12.70
4N6_Qiagen_4b	4.62	9.18

4N6 flocked swabs – ChargeSwitch (Invitrogen)

Assay	Ct SYBR	ng DNA/ µl
4N6_ChSw_1a	9.68	0.36
4N6_ChSw_1b	9.47	0.42
4N6_ChSw_2a	10.46	0.22
4N6_ChSw_2b	10.41	0.23
4N6_ChSw_3a	16.44	0.01
4N6_ChSw_3b	18.72	0.001
4N6_ChSw_4a	12.06	0.08
4N6_ChSw_4b	16.57	0.01

Assay summary

- DNA Micro kit (Qiagen) extraction chemistry provides the best overall yield from 3 extraction methods tested.
- The performance of the DNA IQ (Promega) and ChargeSwitch (Invitrogen) chemistry is practically the same. Both extraction procedures can be performed automatically on the Eppendorf epMotion 5075 LH.
- Sample release efficiency measured for the traditional ryon fiber winded swab achieved accross the extraction protocols on average only 34% of the flocked swabs release capacity.

Methods

Sample collection
Buccal swabs were collected from a single volunteer using the sterile, human DNA-free 4N6 DNA swabs (Copan code number 3520CF) and traditional ryon fiber winded swabs (Copan).

DNA isolation
DNA from samples was extracted using the standard extraction kit chemicals.
Extraction after the lysis step for DNA IQ and ChargeSwitch was performed using the validated method developed for the automated DNA extraction on epMotion 5075 LH automated liquid handling workstation (Eppendorf).
DNA Micro kit extraction was performed manually according to the manufacturer s recommendations.

Real-Time qPCR
Extracted DNA was quantified using the validated quantitation SYBR/ALU method on Mastercycler ep realplex (Eppendorf).

Conclusions

Approximately 1/2 of the biological material (DNA) captured on the flocked swabs is released during 2 minutes in the extraction buffer and so it is not necessary to perform the centrifugation step in the spin baskets and the preparation of samples is faster and less prone to contamination.

The described process of extraction provides sufficient amount of DNA necessary for all down stream forensic DNA identification applications.

The results of experiments proved the expected high efficiency of sample release from the flocked swabs in different lysis buffers and thus verified the suitability for forensic use.

Acknowledgments
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