EVALUATION OF THE INNO-LIPA® HPV GENOTYPING *EXTRA* II ON UCM PRESERVED FIRST-VOID URINE

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INTRODUCTION

Self-sampling for HPV DNA detection opens opportunities to increase the participation rates in screening programs and may improve today's cervical cancer prevention. Non-invasive urine collection is considered the preferred way of self-sampling compared to vaginal/cervical swabs for example by its ease of use. The INNO-LiPA® HPV Genotyping *Extra* II (AMP + LiPA) allows identification of 32 HPV genotypes and excellent performance has been shown using cervical scrapes. Our study aim was to develop a protocol for DNA extraction from first-void urine (FVU) and to evaluate this with the INNO-LiPA® HPV Genotyping *Extra* II in comparison to cervical samples.

METHODS AND MATERIALS

Samples:
Group 1: UCM preserved FVU samples collected with a Colli-Pee™ device (Novosanis, Belgium) between 2010-2012 and stored at -35°C until analysis.
Group 2: paired FVU (Colli-Pee™ - collected) and cervical material (collected with L-shaped endo/esocervical swab (Copan) in 20 mL PreservCyt solution (Hologic))

Testing:
Group 1: DNA was extracted from FVU samples using the QIAamp DNA Mini Kit (Qiagen, Germany) where the standard procedure was applied (i.e. 200 µL sample volume), except for lysis incubation for 30 minutes at 56°C and a final elution volume of 50 µL. Amplification and LiPA hybridization were performed according to the manufacturer's instructions. HPV genotyping results for cervical samples and positivity (only for HPV 16 and 18) in FVU were available from previous studies[1, 2].
Group 2: DNA was extracted from cervical material using the QIAamp MinElute Kit as described in the package insert of the INNO-LiPA® HPV Genotyping *Extra* II Amp Kit. DNA from the corresponding FVU was extracted as described above. Amplification and LiPA hybridization were performed according to the manufacturer's instructions (using Auto-LiPA48).

RESULTS AND DISCUSSION

The HPV genotyping results for group 1 samples compared to historical data are given in Table 1.

The 68 UCM-preserved FVU samples were evaluated with INNO-LiPA® HPV Genotyping *Extra* II starting from 200 µL of sample volume. For 3 samples (5%) a negative result was obtained versus the historical data on FVU starting from 4 mL of sample volume and for 7 samples (10%) other HPV genotypes than HPV 16 or HPV 18 were detected. The observed concordance versus type-specific PCR for HPV 16 or HPV 18 on 4 mL of FVU was 85% and the observed concordance versus HPV genotyping results on cervical material (at least 1 similar HPV type detected) was 87%. However, concordances observed in this group of cervical and 2 different starting volumes of urine samples is done by comparing results from different assays. As DNA degradation cannot be completely excluded, and comparative data was based on historical data, a second study was executed using recently collected samples.
This second study was done using paired cervical and FVU samples. In this case, a true comparison of INNO-LiPA® HPV genotyping of urine samples and cervical samples is performed and its accuracy on urine samples relative to cervical samples (considered as gold standard) can be evaluated.

Table 2 shows the HPV genotyping results for group 2 samples (paired cervical and FVU).

The performance of the INNO-LiPA® HPV Genotyping *Extra* II was evaluated on 30 paired patient samples (cervical material and FVU). In 2 cases (M022 and M067) a negative result was obtained in one of both sample types while HPV X or a LR was detected in the corresponding sample. For all 28 remaining samples at least 1 identical genotype was found in both sample types. For 1 patient sample (M030), HPV 18 was detected in the cervical sample as a co-infection with GT 31, but only GT 31 was observed in the corresponding urine sample. Overall, INNO-LiPA® HPV Genotyping *Extra* II results showed a concordance of > 95% in paired cervical and first-void urine samples.

Table 1: Group 1 HPV genotyping results (sample volume for urine extraction indicated)

Sample	HPV types (cervical)	Urine 16/18 (4 mL)	Urine LiPA (200 µL)	Sample	HPV types (cervical)	Urine 16/18 (4 mL)	Urine LiPA (200 µL)
8	18	+	18	9	16, 31, 39, 53	+	16, 31, 51, 73
6	16	+	HPV X	14	16	+	-
12	18	+	18	15	18	+	18, 53
24	16	+	16	16	16	+	16
30	16	+	16, 89	17	16	+	16
32	16	+	16	19	16, (33,52,58,67)	+	16
38	16	+	16	21	16, 39	+	54, 62
39	16	+	16	22	16	+	-
41	16	+	16	23	neg	-	-
44	16, (33, 52, 58, 67)	+	16, (18), 82	25	16,39, (33, 52, 58,67)	+	16, 33, 39
47	16	+	16	26	16	+	16, 53
49	16	+	16	27	16	+	16, 66, 68
52	16	+	16	28	16	+	16
53	18	+	(18)	29	16	+	16
56	16,35	+	16	31	16	+	16
57	16,35	+	16	34	16	+	16
60	18	+	18, 52, 53	36	16	+	16
61	18	+	18, 89	40	16	+	16, 70
63	16,35	+	16, 35, 61, 81	43	16	+	-
65	16	+	16, 61	54	16	+	16, 70
66	16	+	16, 70	55	16	+	16
69	16,35	+	16	62	16, 53, 59	+	16, 53
78	16	+	16	67	16, 53	+	53
84	16,(33,52,58,67)	+	82	68	16	+	16
85	16	+	16	72	16	+	16, 61, 81
90	16	+	16	73	16	+	16
93	16	+	16	79	16	+	16
97	16	+	59, 61	81	16, 33, 39, (33, 52, 58, 67)	+	16, 33
103	18	+	HPV X	86	16,31, (33, 52, 58, 67)	+	16, 52, 70
106	16	+	16	88	16	+	16, 61
1	18	+	54	95	16	+	16
3	18,39	+	18, 62	96	16	+	16
5	58, (33,52,58,67)	-	-	99	18	+	18, 53
7	16	+	16	100	16	+	16, 51

Table 2: Group 2 HPV genotyping results (red: HR, orange: pHR, green: LR)

Sample	Cervical	Urine	Sample	Cervical	Urine
M012	56, 59	56, 59	M057	16, 33, 53	16, 31, 33, 53
M013	58, 59, 53	58, 68, 53	M060	16, 51	16, 51
M016	68	68	M063	16	16
M018	53	53, 44	M064	16	16
M020	73	73	M066	31	31, 66
M021	53, 82	53, 82	M067	NEG	44
M022	X	NEG	M068	16, 51	16
M023	16	16, 81	M071	52	52
M028	66	66	M073	16	16
M030	18, 31	31	M074	16	16, 89
M031	18, 6	18, 59	M075	45	45
M032	NEG	NEG	M079	61	61
M033	16	16	M081	66	66
M035	45, 68	45, 68	M088	35	35, 82, 83
M047	59	59	M098	81	81

[1] Vorsters et al. 2014. Optimization of HPV DNA detection in urine by improving collection, storage, and extraction. Eur J Clin Microbiol Infect Dis 33:2005-14.

[2] Vorsters et al. 2016. Long-term follow-up of HPV infection by urine and cervical quantitative HPV DNA testing. Int J Mol Sci 17, 750.

CONCLUSION

Initial feasibility data demonstrate that the INNO-LiPA® HPV Genotyping *Extra* II assay can be used on UCM-preserved FVU. HPV DNA genotyping on paired cervical and FVU samples resulted in > 95% concordant results. The high analytical sensitivity of the assay allows reduction of the sample volume and could eliminate the need for a concentration step before extraction. The obtained data suggest that with optimization and standardization of the pre-analytical handling protocol almost equivalent performance of the INNO-LiPA® HPV Genotyping *Extra* II can be achieved both on cervical and FVU samples.