Detection of HPV in first-void urine using an E6/E7 HPV mRNA assay in women referred for colposcopy

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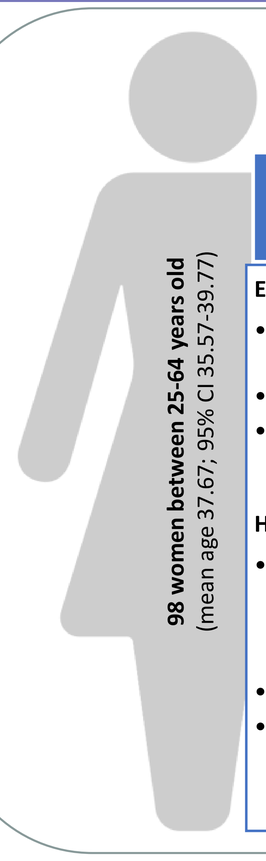
Objective

High correlates between HPV DNA in first-void urine (FVU) and cervical samples have been found. However, because detecting E6/E7 HPV mRNA in cervical smears has proven to be as sensitive as HPV DNA testing, delivering fewer false positive results, this study seeks to elaborate on the detection of E6/E7 HPV mRNA in FVU.

Methods

- Women referred for colposcopy (NCT02714127) were asked to collect a FVU sample (Day 0) (Colli-Pee™, Novosanis) prior to their visit with the gynecologist for a cervical smear and colposcopy.
- Cervical smear and colposcopy results (w/ biopsy if indicated) originate from samples taken at Day 0 ± 3 months.



First-void urine (FVU)

E6/E7 HPV mRNA (n=98)
- 2ml fresh FVU added to an APTIMA® Urine Specimen Transportation Tube (Gen-Probe)
- Aptima® HPV assay (Hologic, Panther)
- HPV**16/18/31/33/35/39/45/51/52/56/58/59**/66/68[a]

HPV genotyping (n=98)
- 4ml FVU + Urine Conservation Medium (2:1 ratio) → Amicon filtration (Merck Millipore) followed by NucliSENS® easyMAG® DNA extraction (bioMérieux) [1]
- Riatol qPCR HPV genotyping assay [2]
- HPV6/11/**16/18/31/33/35/39/45/51/52**/53/**56/58/59**/66/67/68[a]

Cervical smear

Liquid based cytology (LBC) (n=96)
- ThinPrep® Pap Test (Hologic) – Shandon™ /PreservCyt® collection medium (Thermo Fisher/Hologic)
- Nomenclature: Bethesda classification

HPV genotyping (n=95)
- Riatol qPCR HPV genotyping assay [2]
- HPV6/11/**16/18/31/33/35/39/45/51/52**/53/**56/58/59**/66/67/68[a]

Colposcopy, w/ biopsy if indicated

Colposcopy (n=93)
- Performed by 2 gynecologists from the Antwerp University Hospital (UZA)
- Nomenclature: normal, low-, high-grade abnormal lesion

Histology (n=35)
- UZA, pathology
- Nomenclature: CIN (Cervical Intraepithelial Neoplasia) grade 0 (negative) / 3+

[a]High-risk HPV types according to the HPV classification from the International Agency for Research on Cancer (IARC) 2012 are displayed in bold (class 1). HPV68 is classified as probably high-risk (class 2A), HPV66/67 as possibly high-risk (class 2B).

Results

- N=98 paired E6/E7 HPV mRNA (Aptima® HPV assay) and HPV DNA (Riatol qPCR HPV genotyping assay) results in FVU samples (18.73ml (95% CI: 18.49-18.98ml)).
- HPV was detected in 40.82% (E6/E7 HPV mRNA in FVU; n=40/98), 74.49% (HPV DNA in FVU; n=73/98), and 75.79% (HPV DNA in cervical smear; n=72/95) of the samples*.
- A reasonable agreement (kappa value: 0.372 (95% CI: 0.064-0.680)) was found for the Aptima® HPV assay in FVU samples and CIN2+ lesions.
- Sensitivity and specificity values of the Aptima® HPV assay with reference to HPV DNA, liquid based cytology (LBC), colposcopy class, and histology results are illustrated in the table below.

		E6/E7 HPV mRNA (FVU)			Kappa (95% CI)	Chi-Square	Sensitivity (95% CI)	Specificity (95% CI)
		Neg.	Pos.	*Total*				
HPV DNA (FVU)*	Neg.	23	2[§]	*25*	0.307 (0.168-0.446)	**<0.001**		
	Pos.	35	38	*73*				
	Total	*58*	*40*	*98*				
HPV DNA (cervical smear)*	Neg.	21	2[§]	*23*	0.275 (0.138-0.412)	**<0.001**		
	Pos.	36	36	*72*				
	Total	*57*	*38*	*95*				
LBC (≥ASC-US)	Neg.	35	16	*51*	0.199 (-1.761-2.159-	**0.049**	51.11 (35.77-66.30)	68.63 (54.11-80.89)
	Pos.	22	23	*45*				
	Total	*57*	*39*	*96*				
Colposcopy class (≥LSIL)	Neg.	16	7	*23*	0.078 (-0.073-0.229)	0.324	42.03 (30.24-54.52)	69.57 (47.08-86.79)
	Pos.	40	29	*69*				
	Total	*56*	*36*	*92*				
Colposcopy class (≥HSIL)	Neg.	49	27	*76*	0.139 (-0.043-0.321)	0.123	56.25 (29.88-80.25)	64.47 (52.66-75.12)
	Pos.	7	9	*16*				
	Total	*56*	*36*	*92*				
Histology (CIN2+)	Neg.	12	5	*17*	0.372 (0.064-0.680)	**0.028**	66.67 (40.99-86.66)	70.59 (44.04-89.69)
	Pos.	6	12	*18*				
	Total	*18*	*17*	*35*				
Histology (CIN3+)	Neg.	14	11	*25*	0.132 (-0.170-0.434)	0.471[¥]	60.00 (26.24-87.84)	56.00 (34.93-75.60)
	Pos.	4	6	*10*				
	Total	*18*	*17*	*35*				

[§]Samples positive for E6/E7 HPV mRNA (FVU) and negative for HPV DNA in FVU and cervical smears (CS) originate from 4 different women:

FVU-/CS+:

LBC: NILM; Colposcopy: LSIL; Histology: /; HPV DNA: HPV39

LBC: LSIL; Colposcopy: HSIL; Histology: CIN2; HPV DNA: HPV52

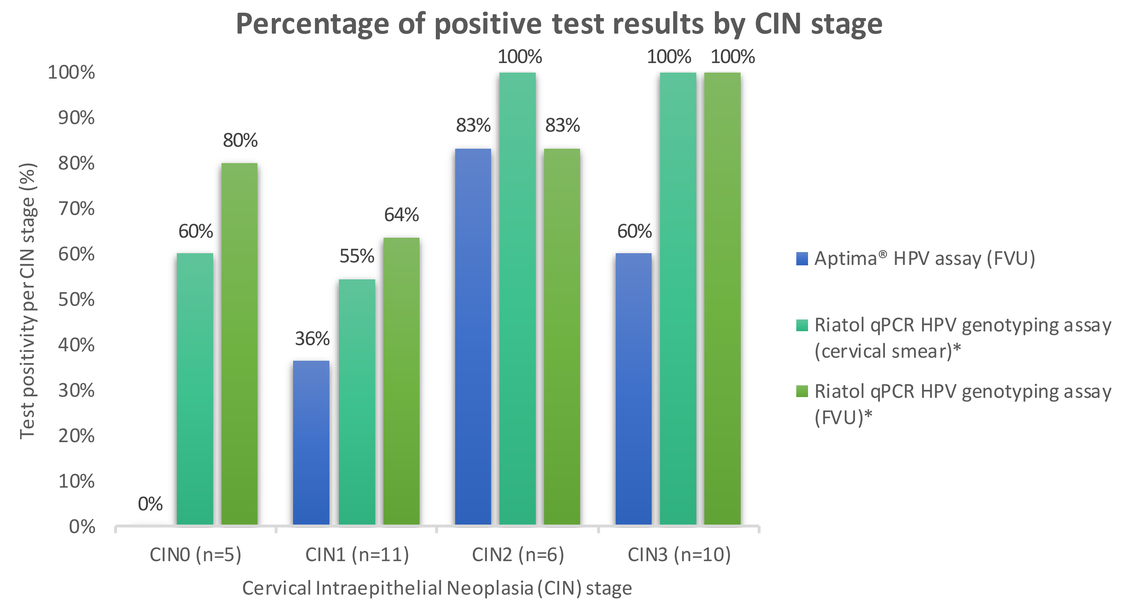
FVU+/CS-:

LBC: NILM; Colposcopy: normal; Histology: /; HPV DNA: HPV33/68

LBC: ASC-US; Colposcopy normal; Histology: CIN0; HPV DNA: HPV45/59/66

*The Riatol qPCR HPV genotyping assay was scored test positive solely for the HPV types included in the Aptima® HPV assay (HPV16/18/31/33/35/39/45/51/52/56/58/59/66/68). **Statistically significant associations** are displayed in purple (Chi-square test; p-value < 0.05). ¥ The fisher exact test was used to correct for >20% of cells with an expected count less than 5. Biopsies where both CIN1 and 2, or CIN2 and 3 lesions were identified were classified as respectively CIN2 and CIN3.

- Histology results are available for 35/98 women, from whom 32 paired FVU, cervical smear, and histology results are available (figure below).



*The Riatol qPCR HPV genotyping assay was scored test positive solely for the HPV types included in the Aptima® HPV assay (HPV16/18/31/33/35/39/45/51/52/56/58/59/66/68). Biopsies where both CIN1 and 2, or CIN2 and 3 lesions were identified were classified as respectively CIN2 and CIN3.

Conclusion

- This study illustrates that **it is feasible to detect E6/E7 HPV mRNA in first-void urine** samples from 25 to 64 year old women referred for colposcopy in Belgium.
- Further study is required to evaluate the clinical performance of the Aptima® HPV assay in FVU samples.

References

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2. Micalessi IM, Boulet GAV, Bogers JJ, Benoy IH, Depuydt CE. High-throughput detection, genotyping and quantification of the human papillomavirus using real-time PCR. Clin Chem Lab Med. 2012;50(4):655-61.

Acknowledgements & Conflicts of interest

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A. Vorsters and P. Van Damme are co-founders of Novosanis (Belgium).

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