Compatibility of a single closed tube real-time PCR assay for testing first void urine specimens.

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Introduction and objectives:

- HPV testing in first void urine has been proposed for monitoring impact of HPV vaccination, follow-up of treatment and/or reaching women not participating in cervical cancer screening programmes.
- Use of first void urine collection device Colli-Pee™ (Novosanis, Belgium) and UCM (Urine Collection Medium, UAntwerp, Belgium) has enhanced the analytical detection of HPV DNA in female urine.
- Anyplex™ II HPV HR assay (Seegene, Korea) can genotype all 14 high risk HPV types in a single closed tube real-time PCR reaction.
- This assay has been previously evaluated on liquid based cytology (LBC) cervical screening samples but not on urine samples for HPV detection.
- **The aim of this pilot study is to determine whether Seegene Anyplex™ HPV HR assay is compatible with self-collected first void urine specimens.**

Methods:

- Study population: 22 women with self-reported prior HPV positive test result
- 176 first void urine samples (from 22 women), were collected using either the Colli-Pee™, first void urine collection device (n=88), or directly into a urine cup (n=88). One participant provided insufficient volume for the urine cup. So the total number of samples processed is 172. Participants alternated the collection times (morning and late afternoon) over 4 consecutive days.
- Samples were collected by the participants at home and were sent by mail at ambient temperature to the University of Antwerp.
- Sample collection and processing is shown in figure 1; prior to the PCR tests, 4ml of urine/UCM mixture was concentrated on an ultrafiltration membrane and extracted with easyMag® (bioMérieux).

Results:

- Good to very good kappa values of 0.682 and 0.817 of the Anyplex™ II HPV HR assay with Riatol qPCR HPV genotyping assay and Optiplex HPV genotyping kits respectively for the HR HPV genotypes (table 1).
- For the Riatol qPCR HPV genotyping assay/Anyplex™ II HPV HR assay and Optiplex HPV genotyping assay/Anyplex™ II HPV HR assay an agreement of 86% and 93% was observed respectively.

Table 1: Agreement between Anyplex™ II HPV HR, Optiplex HPV genotyping and Riatol qPCR HPV genotyping assay.

Type HPV DNA // Alternative assay	Anyplex™+ Alternative+	Anyplex™+ Alternative-	Anyplex™- Alternative+	Anyplex™- Alternative-	Kappa (CI95%CI)
Anyplex™ II HPV HR// Riatol qPCR HPV*	106	19	4	42	0.682 (0.568– 0.796)
Anyplex™ II HPV HR //Optiplex HPV	122	3	9	38	0.817 (0.717 – 0.917)

*one sample failed Riatol qPCR HPV genotyping assay

Table 2: Effect of sampling time and method for signal strength of the internal control (results shown as percentage. 84 samples in each category).**

Signal strength* internal control		++	+++	Total
Time sampling	Afternoon	8.3%	91.7%	N=84
	Morning	9.5%	90.5%	N=84
Method sampling	Colli-Pee™	6.0%	94.0%	N=84
	Urine cup	11.9%	88.1%	N=84

* Signal strength is based on positivity at different melting steps and indicated by plus signs. If sample is positive after 30 cycles it is rated +++. if it is positive after 40 cycles ++. and finally if it is positive after 50 cycles it is rated +.
** Samples having only Colli-Pee data are omitted, total number of samples analyzed is 168.

Conclusions:

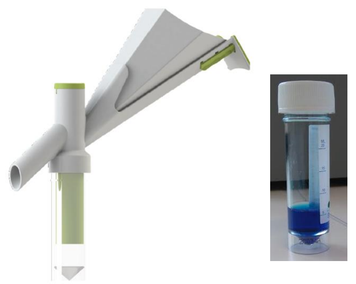
- These preliminary results confirm that the Anyplex™ II HPV HR assay is compatible with self-collected first void urine.

References:

1) A. Vorsters, et al. Optimization of HPV DNA detection in urine by improving collection, storage, and extraction. Eur J Clin Microbiol Infect Dis. 2014
2) Vorsters A, et al. HPV DNA detection in urine: Effect of a first-void urine collection device and time of collection. In: 30th International Papillomavirus Conference. Lisbon. Portugal; 2015.

Figure 1: Sample Collection and Workflow

Samples of 22 women collected in the morning (first urine of the day) and afternoon on 4 consecutive days. while alternating the collection method, were analyzed (n = 8 samples/women* 22 women = 176** samples).



**One participant provided insufficient volume for urine cup samples => total number of samples processed : 176-4 = 172)

Colli-Pee™: Allows capturing fixed volume of guaranteed first void urine and immediate mixing with preservative buffer.

Urine cup: Variable volume of presumed first void and manual transfer to vial with preservative buffer/dye.

Manual Amicon filtration[1]: 4 ml loaded on filter

HPV DNA extraction

NucliSENS® easyMag® extraction. DNA eluted in 55 µl elution buffer

Anyplex™ II HPV HR assay. 5 µl per PCR reaction

Riatol qPCR HPV genotyping assay at AML (1µl per PCR reaction). 35 µl DNA extract is diluted with 40 µl elution buffer.

Optiplex HPV genotyping kit (DiaMex.Heidelberg). 10 µl per PCR reaction

Table 3: Effect of sampling time and method to compare signal strength of the HPV infections (percentage of samples giving total signal strength)

Signal strength HPV types **		0	1	2	3	4	5	6	7	8	Total
Time sampling	Afternoon	23.8%	10.7%	23.8%	10.7%	17.9%	4.8%	3.6%	2.4%	2.4%	N=84
	Morning	28.6%	8.3%	26.2%	7.1%	16.7%	6.0%	2.4%	4.8%	0.0%	N=84
Method sampling	Colli-Pee™	25.0%	9.5%	26.2%	6.0%	16.7%	7.1%	4.8%	2.4%	2.4%	N=84
	Urine cup	27.4%	9.5%	23.8%	11.9%	17.9%	3.6%	1.2%	4.8%	0.0%	N=84

** Total signal strength is provided counting all plus signs for all detected HPV genotypes in one sample. eg. a sample having HPV16(+++). HPV45(++) and HPV35(+) gets a total signal strength of 6.

Discussion:

- Results obtained by Anyplex™ II HPV HR assay are in line with previous data[2]. A good agreement is found with other commercial methods.
- Head to head comparison between assays may not be appropriate as the extraction method also has an impact. Riatol qPCR HPV genotyping assay is performed on more diluted DNA extracts as indicated in figure 1.
- Based on signal strengths, the Colli-Pee ™ collected urine seems to provide higher concentrated urine. but differences were statistically not significant.
- A limitation of this study is that repeated samples from a limited number of subjects are analysed. Larger studies are required to demonstrate performance of the assay.

Disclosure:

Novosanis is a spin-off company of the University of Antwerp. VA, VV, BK, and VDP are co-founders and board members of Novosanis.

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