# Performance of an Automated HPV Genotyping Assay using First Void Urine Specimens

Alex Vorsters[1], Vanessa Vankerckhoven [1,2], Manon de Koeijer[2], Koen Beyers[2], Severien Van Keer[1], Samantha Biesmans[1], Pierre Van Damme[1], Danyelle Long[3], Ryan Schwab[3], Erin Gutierrez[3], William Nussbaumer[3], James Harris[3], Laurence Vaughan[3], [1]University of Antwerp, VAXINFECTIO, Belgium, [2]Novosanis, Belgium, [3]BD Diagnostics, USA

## Introduction and objectives:

HPV testing from self-collected first void urine specimens is an attractive option in both high and low resource settings. We examined the performance of the BD Onclarity™ HPV Assay in women with a history of high-risk HPV infection*.

*Urine is not an approved sample type for the BD Onclarity™ HPV Assay and its performance characteristics have not been established.

## Methods:

First void urine samples were self-collected by women at home using a standard urine cup or the Colli-Pee™ (Novosanis), a first void urine collection device[1]. Collection was done in the morning and later in the day on 4 consecutive days from the same women, while alternating the collection methods. Urine samples were then mailed at ambient temperature to the test laboratory. Results from 0.8 mL of Onclarity-extracted urine/UCM preservative mixture tested on the BD Onclarity Assay on the automated BD Viper™ LT System were compared to results from 4 mL urine/UCM preservative mixture concentrated on an ultrafiltration membrane and extracted with NucliSENS easyMag® (bioMérieux) prior to testing with an E6/E7 TS real time genotyping assay (RIATOL).

Figure 1. Sample Collection and Workflow

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

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

Samples of 21 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* 21 women = 168 samples).

Two different HPV DNA extraction and detection methods

Manual Amicon filtration: 4mL loaded on filter

NucliSENS® easyMag® extraction



TS realtime PCR at RIATOL, Antwerp Belgium

"Load and Go" Automated Workflow

0.8 mL automated extraction

Automated Extration, Amplification and Results Output

BD Viper™ LT System

| | | |
|---|---|---|
| **Time to Result:** | **4.16 hours** | **2.50 hours** |
| **Hands on time:** | **2.33 hours (20 samples)** | **0.25 hours (30 samples)** |

## Results:

Performance using the membrane captured semi-automated workflow and the fully automated BD Onclarity™ workflow were very similar (Table 1). The individual viral PCR cycles (Ct values) for the two collection methods across each patient is shown in Figure 2. Both assays showed good reproducibility in detecting both human and viral DNA. The average Ct value for human beta globin gene detection for Onclarity was 27.3 which is approximately 4-fold less than a typical liquid-based cytology (LBC) endocervical specimen (Average Ct = 25.0; data not shown). Both single and mixed infections were reliably detected in serially collected specimens from the same patients. The Colli-Pee collection device showed a better detection by the Onclarity assay i.e. lower Ct values ($p < 0.0001$) and more positive signals (Figure 2 and 3) than the urine cup. A higher kappa value between both detection methods is observed for Colli-Pee collected urine (Table 1). Ct values were broadly similar for both collection times but we noted a slightly significant improvement in Ct scores between morning and later afternoon samples ($p = 0.037$) using the Onclarity assay.

Table 1. Performance both extraction and PCR detection methods.

| Method | BD+ TS RT PCR+ | BD+ TS RT PCR- | BD- TS RT PCR+ | BD- TS RT PCR- | Kappa** |
|---|---|---|---|---|---|
| Colli-Pee™ Urine Collection | 54 | 5 | 3 | 20 | 0.765 (0.610 – 0.920) |
| Manual Urine Cup and Pipette Transfer | 50 | 6 | 6 | 20 | 0.662 (0.486 – 0.839) |
| Combined | 104 | 11 | 9 | 40 | 0.712 (0.594 – 0.830) |

** Cohen's Unweighted Kappa - indeterminate samples (n = 2 for both methods) were omitted from the analysis

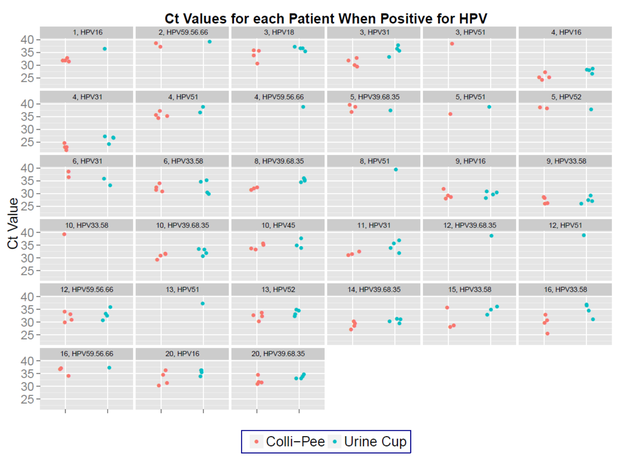


Figure 2. BD Onclarity Ct scores for Colli-Pee™ and Urine Cup specimens from patients positive for HPV

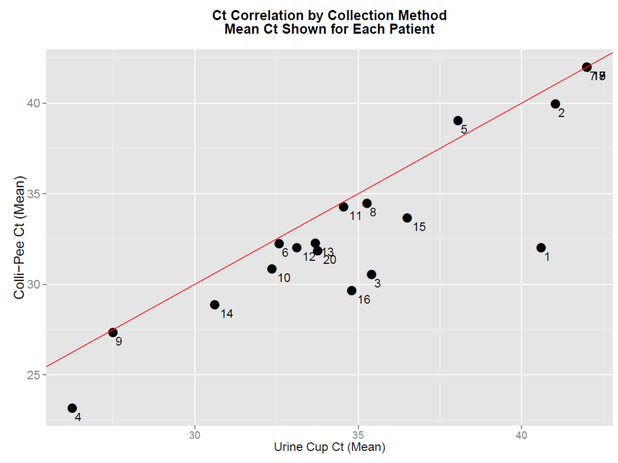


Figure 3. Comparison of patient average Onclarity Ct scores by collection method. The Colli-Pee device-collected urine specimens tend to have lower Ct values

## Discussion:

The BD Viper LT automated assay had similar performance to the semi-automated reference method with a higher throughput and minimal hands on time. The Colli-Pee collection device resulted in a higher analytic sensitivity than the urine cup method. Both assay methods provided robust detection of both human and viral DNA targets. The results suggest that the BD Onclarity™ HPV Assay reliably detects HPV in urine specimens and offers the potential of a high quality and high ease of use sample type for HPV screening. The difference between morning and later in the day urine samples warrants further investigation using a larger sample size.

## Conclusions:

The automated BD Onclarity assay recorded similar results to the manual, labor intensive procedure and confirms recent findings that first void urine offers a promising new approach for self-collected HPV specimens[1]. The small sample volume and fully integrated workflow offer a number of advantages over current methods. Clinical cut-off determination versus CIN2+ histology endpoints will be addressed in future studies.

## Disclosure:

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

## References:

1. A. Vorsters, J. Van den Bergh, I. Micalessi, S. Biesmans, J. Bogers, A. Hens, I. De Coster, M. Ieven, P. Van Damme. Optimization of HPV DNA detection in urine by improving collection, storage, and extraction. Eur J Clin Microbiol Infect Dis. 2014

**Contact:**
alex.vorsters@uantwerpen.be