Evaluation of the Roche COBAS® 6800 HPV assay with Colli-Pee™ collected, UCM preserved Urine.

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Introduction and objectives:

- HPV testing in urine has been proposed for monitoring impact of vaccination, follow-up of treatment and/or reaching women not participating in cervical cancer screening programs.[1]
- Use of Colli-Pee™ (Novosanis, Belgium) and UCM (Urine Collection Medium, UAntwerp, Belgium) has enhanced the analytical detection of HPV DNA in female urine.
- The aim of this pilot study **was to check compatibility with Colli-Pee™ collected, UCM preserved urine and compare performance of the Roche Cobas® 4800 and 6800 HPV assays.** In addition, a pilot on **impact of different preservatives on HPV detection onto the Cobas® 6800 System** was conducted.

Methods:

- 44 Colli-Pee™ collected, UCM preserved urine samples were analysed.
- All samples were characterised by an in-house HPV type specific (TS) qPCR method (UAntwerp, Belgium) and/or by the Optiplex HPV genotyping kit (Diamex, Heidelberg, Germany). Extraction and detection flow are shown in figure 1.
- We further tested 15 urine samples of which 10 were previously positive for HPV 16 and/or 18. These samples were stored for 3 days at ambient temperature without preservative, with UCM or with Roche preservative.

Figure 1. Sample Collection and Workflow

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

Manual Amicon filtration: 4mL loaded on filter

NucliSENS®easyMag® extraction, eluted in 100µl elution buffer

TS realtime PCR; 5µl per PCR reaction; net volume of sample for PCR: 200µl

cobas® 4800 System

0.4 ml used in the automated extraction; elution in 125µl; 25µl used for PCR reaction; net volume of sample for PCR: 80µl

cobas® 6800 System
384 tests/8-hour shift

0.4 ml used in the automated extraction; elution in 50µl; 27µl used for PCR reaction; net sample volume for PCR: 216µl

HPV genotyping DiaMex, 10µl per PCR reaction; net volume of sample for PCR: 400µl

Results:

- Figure 1 shows that the net volume of sample used for the PCR reaction in the different extraction detection systems ranges between 80 and 400µl. This net volume is calculated based on total sample volume used for DNA extraction and the fraction of DNA elution that is used for the PCR reaction.
- All 44 samples were positive for the Roche beta-globin (BG) internal control.
- Compared to Cobas®4800 HPV assay an additional 8 samples were positive for HPV 16 with the Cobas®6800
- Figure 2 and 3 show the correlation between the CT (cycle threshold) values obtained with Cobas® 4800, 6800 HPV and the in-house TS qPCR for human DNA and HPV DNA. Lower CT values for the IC control as well as for the HPV 16 are observed with the Cobas®4800 compared to Cobas® 6800.
- The impact of preservative is shown in table 2 and is most noted at the level of the Internal Control, i.e. 4 of the 15 samples without preservative were reported invalid. Also two HPV 16 results and one ”HPV other” result are reported negative if no preservative is added.

Table 1: Raw data of three different detection methods. (nt: not tested)

Ref	Roche Cobas 4800 HPV			Roche Cobas 6800 HPV			Optiplex HPV genotype DiaMex		In-house TS qPCR		
	HPV Result	HPV (Ct)	BG(CT)	HPV Result	HPV (Ct)	BG(CT)	HPV type	MFI	HPV16 (Ct)	HPV18 (Ct)	GAPDH (Ct)
1	neg	-	31.4	neg	-	29.0	56.0	198.0	nt	0.0	28.1
2	18.0	33.3	28.4	18.0	31.3	26.6	18.0	452.0	nt	35.4	29.0
3	other	35.4	29.1	16+other	34.8+31.5	27.0	16; 33; 42; 51	1638; 385; 44; 95	35.2	nt	26.1
4	other	20.7	27.9	other	17.1	24.9	33; 51; 53; 82	36; 798; 160; 73,5	0.0	0.0	26.7
5	neg	-	31.1	other	36.1	28.0	51; 52; 53	64; 469; 871,5	nt	0.0	27.6
6	other	32.5	28.1	other	28.6	25.5	53; 59	84; 1357	nt	0.0	24.0
7	16+18	37.2+36.0	28.4	16+18	34.3+31.7	26.8	16; 18	2975,5; 493	33.5	34.4	27.6
8	neg	-	26.8	16.0	35.2	24.7	16.0	995.5	35.3	nt	22.6
9	18.0	34.6	30.6	18.0	31.1	29.0	18; 42	531; 455,5	0.0	nt	28.0
10	neg	-	28.2	neg	-	26.5	neg	neg	0.0	nt	27.3
11	16.0	33.4	29.8	16.0	28.7	27.6	16.0	4119.5	27.2	nt	26.6
12	neg	-	29.5	neg	-	27.6	33.0	54.0	0.0	nt	26.9
13	16.0	37.0	27.4	16.0	35.2	25.3	16.0	3450.0	32.4	nt	25.4
14	neg	-	27.6	neg	-	25.1	16.0	1114.0	35.7	nt	25.7
15	16.0	27.5	28.4	16.0	25.8	26.7	11; 16	270; 4201	23.9	nt	27.4
16	neg	-	28.1	neg	-	26.2	16.0	613.5	0.0	nt	25.6
17	neg	-	32.7	16.0	34.3	30.9	42; 52; 70	181,5; 654; 849	0.0	nt	29.8
18	neg	-	26.8	neg	-	24.6	51.0	16.0	0.0	nt	24.3
19	other	37.1	29.6	16+other	35.3+30.5	27.2	16; 39	3405; 641	34.7	nt	27.2
20	other	32.1	29.5	16.0	35.2	28.1	53; 59	966,5 ; 52	0.0	nt	27.1
21	neg	-	27.9	16.0	36.1	25.7	16; 73	2754; 28	31.3	nt	25.0
22	16.0	37.7	28.4	16.0	33.7	25.8	16.0	3403.0	31.9	nt	24.3
23	neg	-	27.7	neg	-	25.2	neg	neg	nt	0.0	24.7
24	neg	-	30.1	neg	-	26.8	neg	neg	0.0	nt	25.8
25	16.0	27.9	29.5	16.0	24.9	25.9	16.0	4548.0	23.1	nt	26.3
26	neg	-	31.4	neg	-	29.2	neg	neg	0.0	nt	28.1
27	16.0	27.9	29.4	16.0	25.0	26.6	16; 44; 53	4308; 180; 225;5	23.6	nt	26.0
28	16.0	35.5	26.9	16.0	32.8	25.1	16.0	3445.0	29.8	nt	24.5
29	neg	-	28.4	neg	-	27.2	neg	neg	0.0	nt	28.3
30	neg	-	32.4	16.0	35.7	30.3	16.0	2772.0	36.2	nt	30.8
31	neg	-	27.4	neg	-	25.2	neg	neg	nt	0.0	24.8
32	neg	-	30.9	neg	-	28.4	neg	neg	0.0	nt	27.7
33	16.0	33.4	28.3	16.0	30.5	26.3	16.0	1559.0	nt	nt	25.7
34	other	28.9	31.2	other	25.4	28.9	51.0	587.5	nt	nt	29.0
35	other	29.3	28.6	other	27.1	25.6	16; 56; 66	31; 364,5; 699	nt	nt	25.1
36	other	34.4	27.9	other	30.6	25.3	39.0	20.0	nt	nt	22.9
37	other	27.7	28.1	other	24.7	25.1	39; 42	217; 37,5	nt	nt	24.6
38	16+other	39.8+35.1	27.1	16+other	35.4+33.9	24.2	16; 42; 45	25; 32; 37	nt	nt	24.4
39	neg	-	33.5	16.0	37.8	31.1	16; 53	140; 97,5	nt	nt	29.1
40	other	39.3	29.5	other	35.9	26.9	56.0	138.0	nt	nt	25.9
41	16.0	35.7	29.4	16.0	32.9	28.0	16.0	2796.0	31.2	nt	23.4
42	other	37.8	32.1	other	33.9	27.5	6; 51; 52	63,5; 465,5; 330,5	0.0	nt	27.5
43	neg	-	30.4	other	30.8	28.2	16; 44; 52; 70	145; 264,5; 453; 1108	0.0	nt	28.9
44	16+other	36.5+31.7	28.9	16+other	33.4+29.7	27.1	16; 39	1684; 1024	33.5	nt	25.7

Figure 2. CT values for human DNA

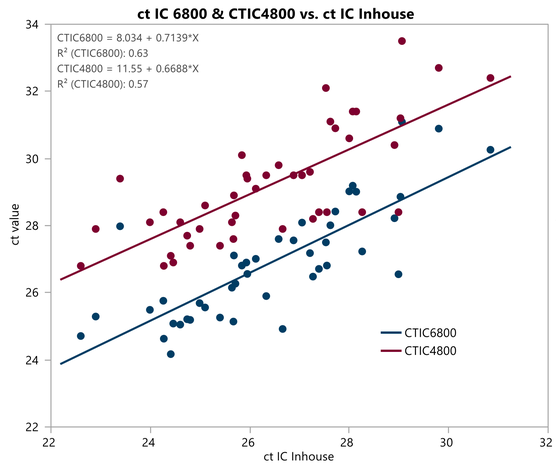

Figure 3. CT values for HPV16

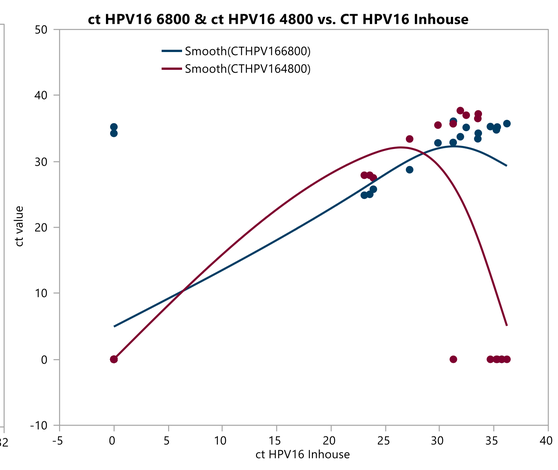

Table 2: Impact of preservative on Ct values of beta globin (BG), HPV16 and HPV18 after 3 days at ambient temperature. (not corrected for dilution with preservative)

Signal	Preservative	Sample ID														
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
IC BG	Roche	29.2	30.6	36.8	30.2	28.5	26.1	34.9	27.3	29.2	27.6	24.9	28.9	30.4	28.8	29.2
	UCM	29.6	30.1	35.1	31.4	30.2	26.3	0.0	27.9	32.8	29.3	25.2	31.2	30.8	30.1	28.6
	No buffer	35.4	0.0	37.0	32.5	34.5	31.4	0.0	33.3	33.8	34.6	30.6	0.0	34.3	0.0	33.6
HPV 16	Roche	0.0	24.2	38.1	36.5	34.1	34.3	31.6	0.0	34.3	37.0	0.0	0.0	0.0	0.0	0.0
	UCM	0.0	23.7	35.8	0.0	35.2	35.1	35.1	0.0	37.2	37.4	0.0	0.0	0.0	0.0	0.0
	No buffer	0.0	24.0	36.6	0.0	36.9	37.1	35.6	0.0	37.6	0.0	0.0	0.0	0.0	0.0	0.0
HPV 18	Roche	0.0	0.0	0.0	0.0	0.0	0.0	0.0	32.2	0.0	29.7	0.0	0.0	0.0	0.0	0.0
	UCM	0.0	0.0	0.0	0.0	0.0	0.0	0.0	32.9	0.0	33.8	0.0	0.0	0.0	0.0	0.0
	No buffer	0.0	0.0	0.0	0.0	0.0	0.0	0.0	35.7	0.0	33.4	0.0	0.0	0.0	0.0	0.0
Other HPV	Roche	0.0	0.0	0.0	34.5	0.0	0.0	0.0	0.0	35.6	21.7	35.1	0.0	0.0	32.8	0.0
	UCM	0.0	0.0	0.0	35.7	0.0	0.0	0.0	0.0	36.7	21.5	34.4	0.0	0.0	32.8	0.0
	No buffer	0.0	0.0	0.0	34.1	0.0	0.0	0.0	0.0	36.4	21.7	35.7	0.0	0.0	0.0	0.0

Conclusions:

- We confirm that the Cobas®6800 HPV assay is compatible with Colli-Pee™ collected, UCM preserved urine.
- The increased net sample volume processed in the qPCR of the Cobas®6800 HPV reflects in an increased analytical sensitivity compared to the Cobas®4800.
- The importance of the use of a preservative is reconfirmed.
- These results are very encouraging to further investigate possible applications of first void urine in combination with the Roche Cobas® HPV assay.

Disclosure:

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

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