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

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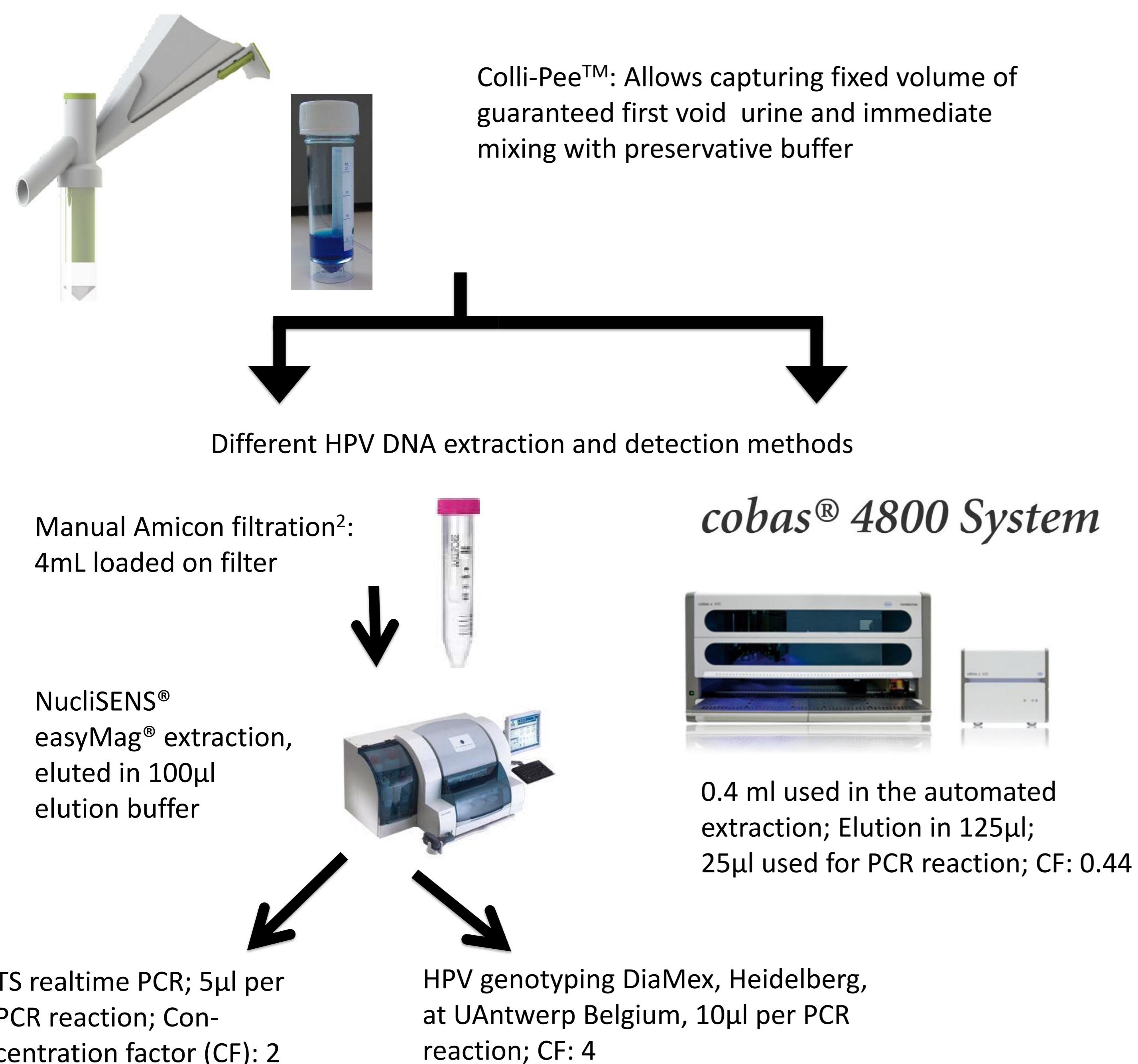
Introduction and objectives:

- 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.
- 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 Roche Cobas®HPV assay has been validated for clinical sensitivity during the ATHENA trial enrolling > 47,000 women on cervical material[1].
- The aim of this pilot study is **to determine if the Roche Cobas®HPV assay is compatible with Colli-Pee™ collected, UCM preserved urine.**

Methods:

- 44 Colli-Pee™ collected, UCM preserved urine samples were analysed:
- 32 originated from a cohort of women participating in a therapeutic HPV vaccination trial – samples were collected by the participants at home and were sent uncooled by mail to the University of Antwerp
- 12 positive samples from previous trials were added; female students (B) and self-reported HPV positive women (C)
- 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.

Figure 1. Sample Collection and Workflow

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

Different HPV DNA extraction and detection methods

Manual Amicon filtration[2]: 4mL loaded on filter

cobas® 4800 System

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

0.4 ml used in the automated extraction; Elution in 125µl; 25µl used for PCR reaction; CF: 0.44

TS realtime PCR; 5µl per PCR reaction; Concentration factor (CF): 2

HPV genotyping DiaMex, Heidelberg, at UAntwerp Belgium, 10µl per PCR reaction; CF: 4

Results:

- All samples were positive for the Roche beta-globin internal control signal (Table 1)
- high positive correlations between CT values obtained by our in-house human DNA (GAPDH) PCR and the Roche beta-globin internal control CT values (Figure 2)
- Very high positive correlation for CT values of HPV 16 DNA (Figure 3)
- The agreement of the COBAS®Roche assay with Luminex based DiaMex assay and our HPV 16/18 in-house PCR is shown in Table 2.

Conclusions:

- We confirm that the Cobas®HPV assay is compatible with Colli-Pee™ collected, UCM preserved urine.
- These results are very encouraging to further investigate possible applications of first void urine in combination with the Roche Cobas®HPV assay.

References:

1) T. Wright, M. Stoler, C. Behrens, A. Sharma, G. Zhang, T. Wright. Primary cervical cancer screening with human papillomavirus: End of study results from the ATHENA study using HPV as the first-line screening test. Gynecologic Oncology. 2015
2) 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
3) G. Stanczuk, G. Baxter, H. Currie, J. Lawrence, K. Cuschieri, A. Wilson, M. Arbyn; Clinical validation of hrHPV testing on vaginal and urine self-samples in primary cervical screening (cross-sectional results from the Papillomavirus Dumfries and Galloway—PaVDaG study); BMJ Open 2016.

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

Ref	Roche Cobas HPV assay			Optiplex HPV genotyping DiaMex		In-house TS qPCR		
	HPV Result	HPV (Ct)	BG (Ct)	HPV type	MFI	HPV16 (Ct)	HPV18 (Ct)	GAPDH (Ct)
C1	OTHER+16	31.7+36.5	28.9	16;39	1684;1024	33.51	nt	25.68
C2	NEG	-	30.4	16;44;52;70	145;264,5;453;1108	0	nt	28.91
C3	OTHER	37.8	32.1	6;51;52	63,5;465,5;330,5	0	nt	27.53
C4	16	35.7	29.4	16	2796	31.24	nt	23.39
B1	OTHER	39.3	29.5	56	138	nt	nt	25.93
B2	NEG	-	33.5	16;53	140;97,5	nt	nt	29.06
B3	OTHER+16	35.1+39.8	27.1	16;42;45	25;32;37	nt	nt	24.41
B4	OTHER	27.7	28.1	39;42	217;37,5	nt	nt	24.6
B5	OTHER	34.4	27.9	39	20	nt	nt	22.9
B6	OTHER	29.3	28.6	16;56;66	31;364,5;699	nt	nt	25.1
B7	OTHER	28.9	31.2	51	587.5	nt	nt	29.03
B8	16	33.4	28.3	16	1559	nt	nt	25.71
A1	NEG	-	30.9	NEG	NEG	0	nt	27.72
A2	NEG	-	27.4	NEG	NEG	nt	0	24.8
A3	NEG	-	32.4	16	2772	36.19	nt	30.84
A4	NEG	-	28.4	NEG	NEG	nt	nt	28.26
A5	16	35.5	26.9	16	3445	29.84	nt	24.46
A6	16	27.9	29.4	16;44;53	4308;180;225;5	23.56	nt	25.95
A7	NEG	-	31.4	NEG	NEG	0	nt	28.07
A8	16	27.9	29.5	16	4548	23.06	nt	26.33
A9	NEG	-	30.1	NEG	NEG	0	nt	25.84
A10	NEG	-	27.7	NEG	NEG	nt	0	24.74
A11	16	37.7	28.4	16	3403	31.9	nt	24.26
A12	NEG	-	27.9	16;73	2754;28	31.26	nt	24.99
A13	OTHER	32.1	29.5	53;59	966,5;52	0	nt	27.05
A14	OTHER	37.1	29.6	16;39	3405;641	34.67	nt	27.21
A15	NEG	-	26.8	51	16	0	nt	24.27
A16	NEG	-	32.7	42;52;70	181,5;654;849	0	nt	29.8
A17	NEG	-	28.1	16	613.5	0	nt	25.64
A18	16	27.5	28.4	11;16	270;4201	23.89	nt	27.39
A19	NEG	-	27.6	16	1114	35.71	nt	25.67
A20	16	37	27.4	16	3450	32.44	nt	25.4
A21	NEG	-	29.5	33	54	0	nt	26.88
A22	16	33.4	29.8	16	4119.5	27.23	nt	26.58
A23	NEG	-	28.2	NEG	NEG	0	nt	27.27
A24	18	34.6	30.6	18;42	531;455,5	0	35.44	28
A25	NEG	-	26.8	16	995.5	35.3	nt	22.6
A26	16+18	37.2+36.0	28.4	16;18	2975,5;493	33.55	34.37	27.55
A27	OTHER	32.5	28.1	53;59	84;1357	nt	0	23.99
A28	NEG	-	31.1	51;52;53	64;469;871,5	nt	0	27.62
A29	OTHER	20.7	27.9	33;51;53;82	36;798;160;73,5	0	0	26.66
A30	OTHER	35.4	29.1	16;33;42;51	1638;385;44;95	35.22	nt	26.12
A31	18	33.3	28.4	18	452	nt	35.44	28.99
A32	NEG	-	31.4	56	198	nt	0	28.14

Figure 2. Correlation CT values for human DNA

R^2 Linear = 0.566

y=11.55+0.67*x

Dotted-line is line of equality; straight line shows linear correlation.

Figure 3. Correlation CT values for HPV16

R^2 Linear = 0.941
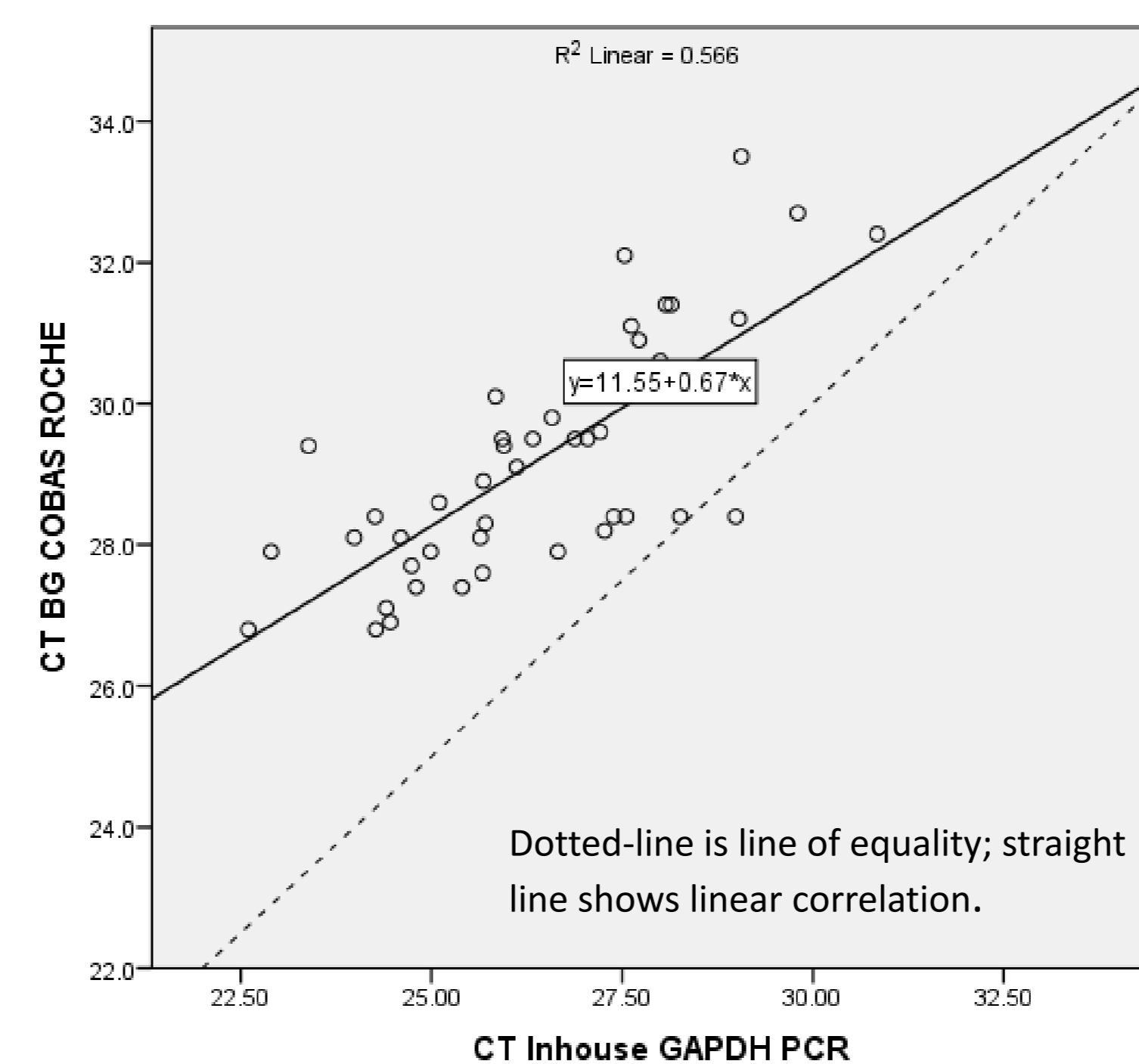
y=5.58+0.97*x

Dotted-line is line of equality; straight line shows linear correlation.
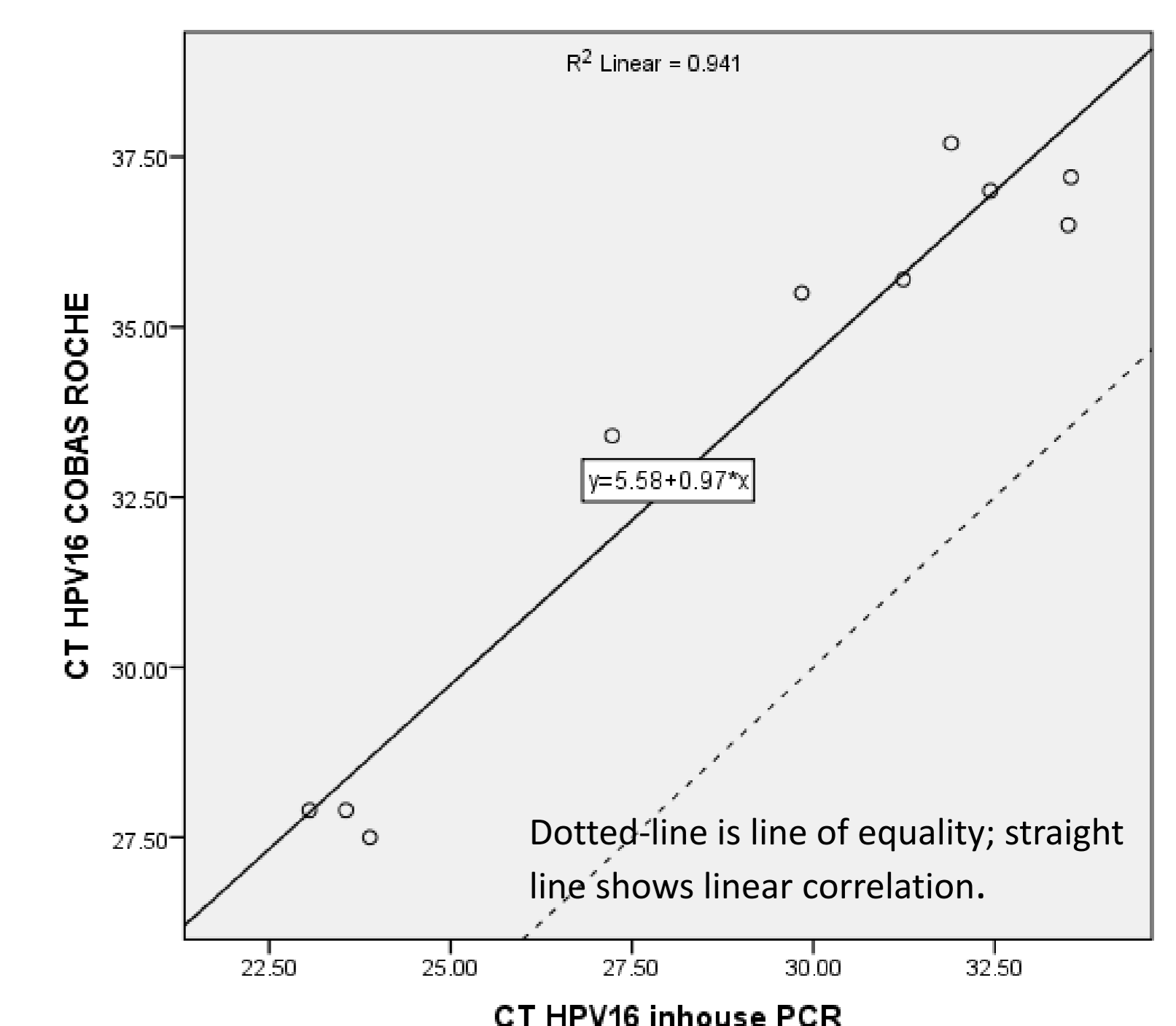
Table 2 Agreement between Cobas Roche, DiaMex and in-house PCR.

Type HPV DNA // Alternative assay	Roche+ Alternative+	Roche+ Alternative-	Roche- Alternative+	Roche- Alternative-	Kappa (CI95%)
All HPV DNA // DiaMex HPV	25	0	12	7	0.399 (0.168 – 0.630)
HPV16/18 // DiaMex HPV	14	0	10	20	0.662 (0.486 – 0.839)
HPV16/18// In house TS qPCR	12	0	6	18	0.667 (0.896 – 0.438)

Discussion:

- In addition to a valid Roche Cobas®internal control signal for all samples we observe a **linear correlation** between the Cobas Roche CT values and the in-house PCR CT values.
- The analytical sensitivity of DiaMex and the in-house HPV 16/18 PCR using amicon concentrated and Nuclisence Easymag extracted DNA is higher: indeed, compared to The Roche Cobas PCR potentially 4.5 to 9.1 times more DNA enters the PCR reaction for respectively the in-house and DiaMex HPV PCR. In addition, the sensitivity of the Roche®Cobas assay has been set for detection of clinical relevant infections in cervical samples.
- Proper collected first void urine and a modified internal threshold may lead to substantial increased sensitivity[3].