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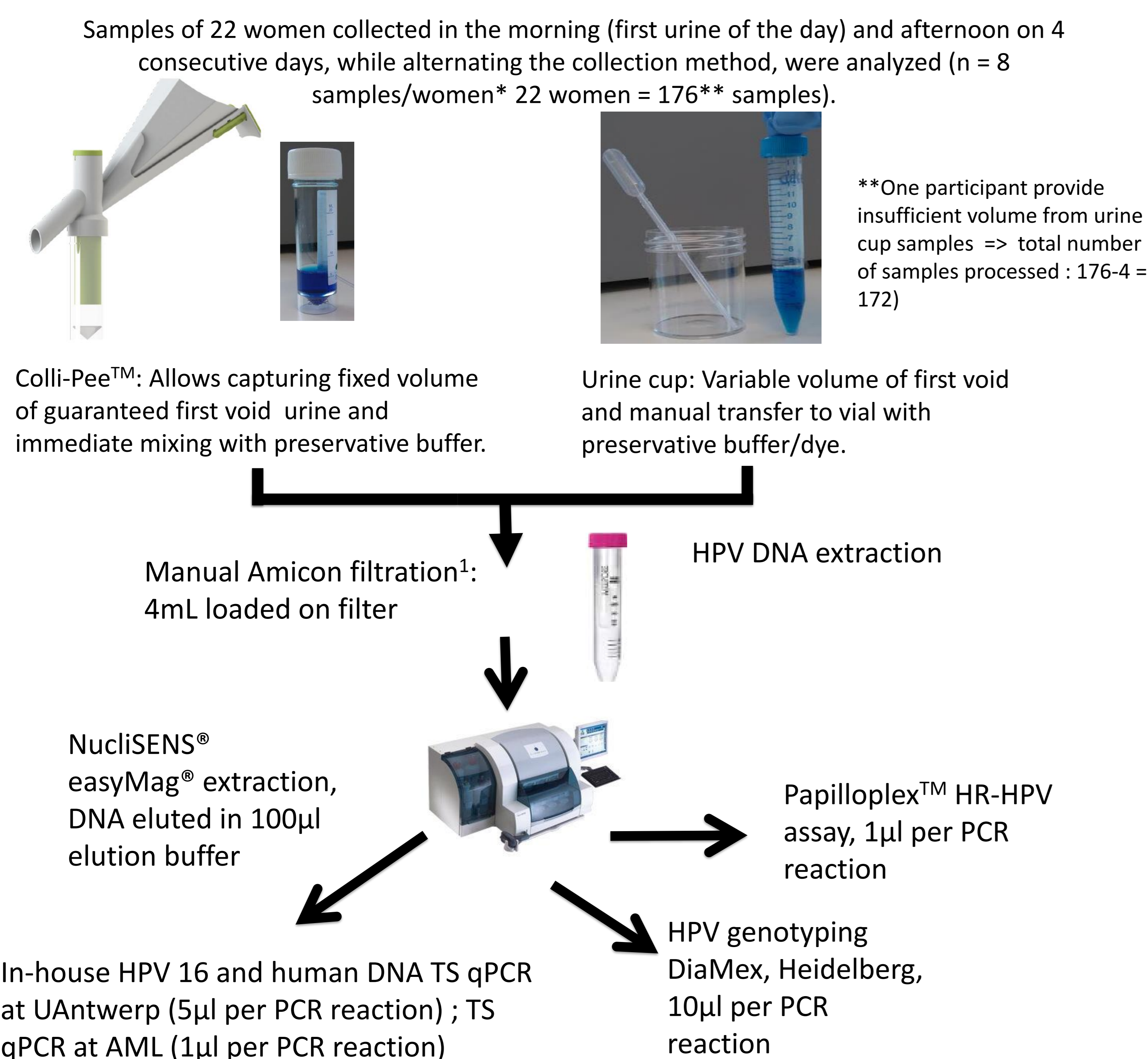
Introduction and objectives:

- HPV urine testing 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
- Genefirst Papilloplex™ HR-HPV assay can genotype and quantify 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 Genefirst Papilloplex™ HR-HPV test's compatibility with self-collected first void urine specimens and to investigate the effect of the sampling method.**

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). Participants alternated the collection times (morning and late afternoon) over 4 consecutive days.
- Samples were collected by the participants at home and were sent uncooled by mail 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).

Figure 1. Sample Collection and Workflow



Results:

- Good agreement of 0.676 and 0.669 of Papilloplex™ HR-HPV assay with AML TS qPCR and Optiplex HPV genotyping kits respectively for the HR HPV genotypes (table 1).
- High positive correlation recorded for the CT values from our in-house human DNA (GAPDH) qPCR and the human DNA control of the Papilloplex™ HR-HPV assay (Figure 2).
- A very high positive correlation for HPV 16 DNA CT values between the two assays observed as in Figure 3.
- Impact of collection method observed in Figure 4 and 5.
- Average CT values reported in Colli-Pee collected urine for human DNA (Figure 4), and HPV DNA (Figure 5) found to be lower than the CT values from urine cup collected samples.

Table 1: Agreement between Papilloplex, Optiplex and the in-house TS qPCR.

Type HPV DNA // Alternative assay	Papilloplex+ Alternative+	Papilloplex+ Alternative-	Papilloplex- Alternative+	Papilloplex- Alternative-	Kappa (CI95%)
All HR HPV DNA // AML TS qPCR	106	14	10	42	0.676 (0.556 – 0.796)
All HR HPV DNA // Optiplex HPV	108	12	12	40	0.669 (0.547 – 0.790)

Figure 2. Correlation CT values for human DNA, Papilloplex versus in-house qPCR.

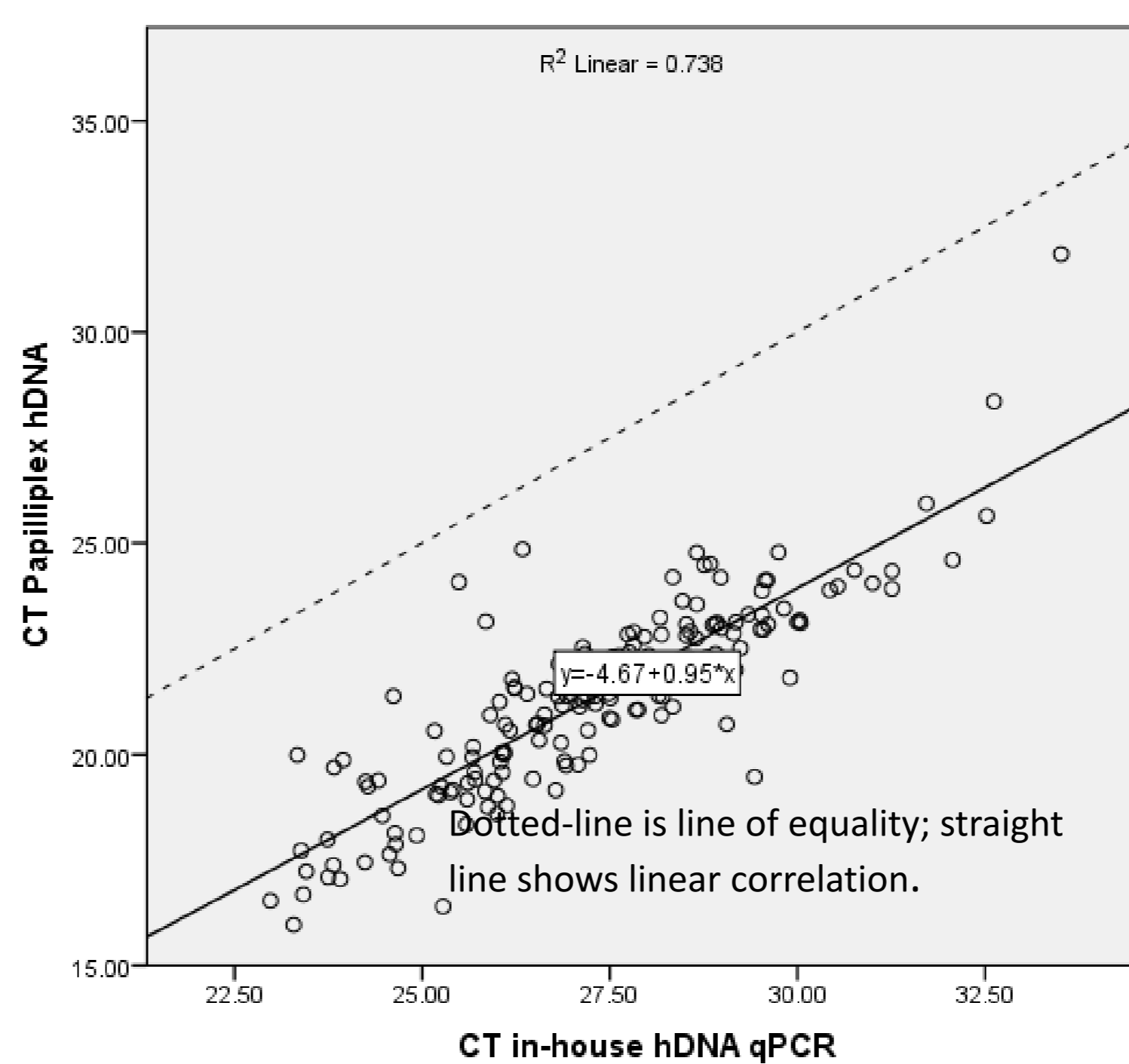


Figure 3. Correlation CT values for HPV16, Papilloplex versus in-house qPCR.

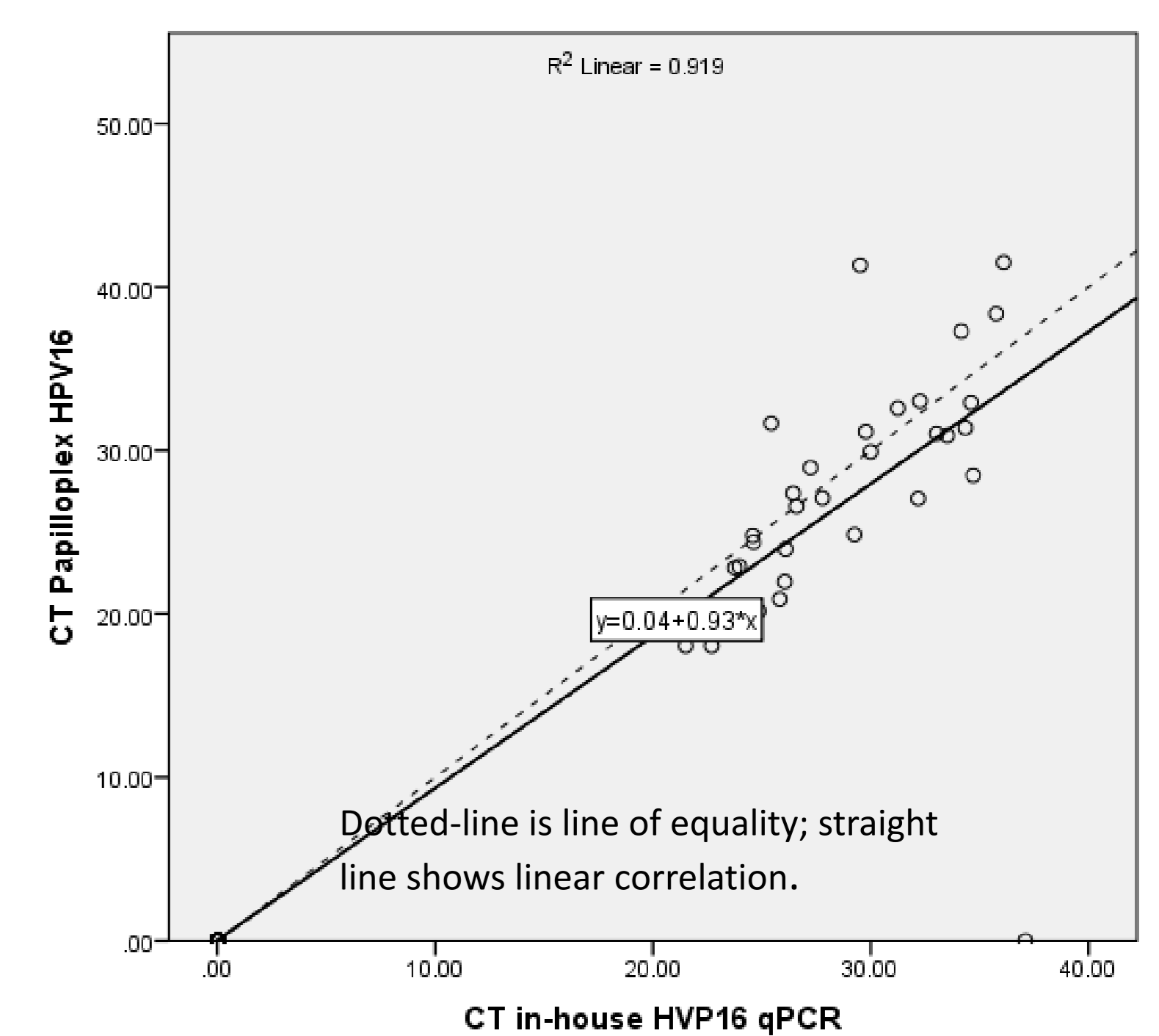


Figure 4. Effect of sampling method CT values for human DNA

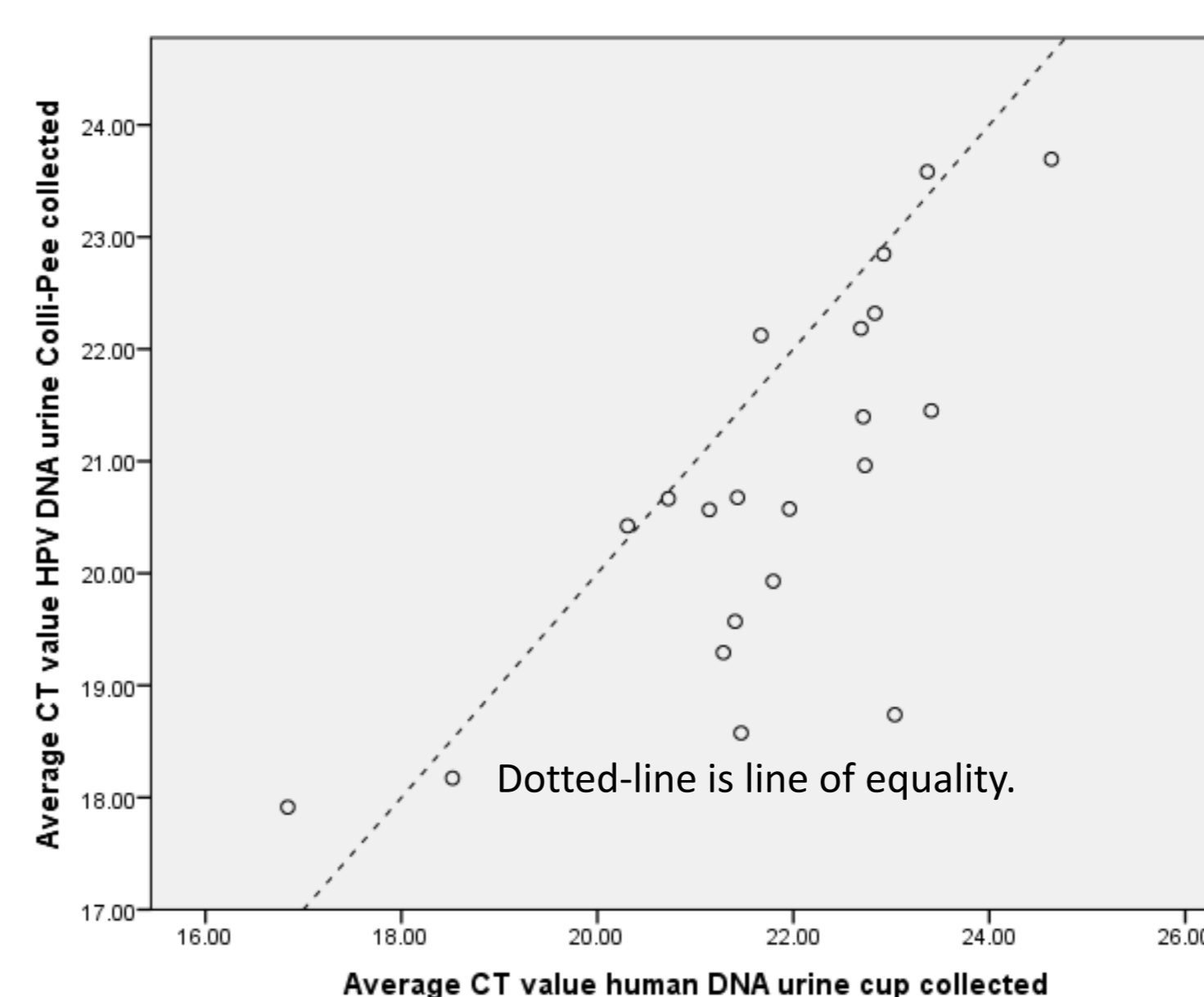
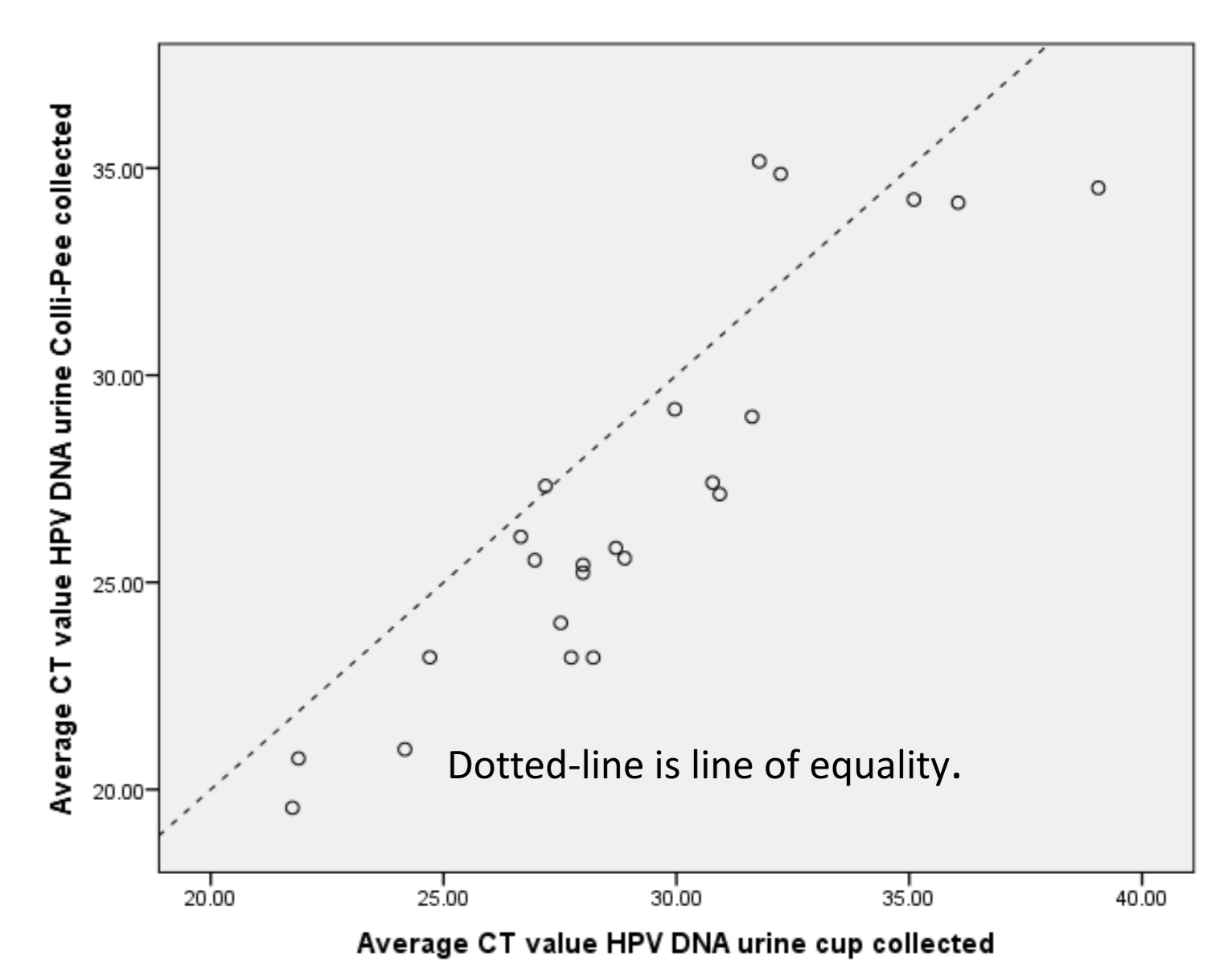


Figure 5. Effect of sampling method CT values for HPV DNA



Discussion:

- Results obtained by Papilloplex™ HR-HPV are in line with previous obtained data². A good agreement is found with other commercial methods.
- The impact of a first void urine sampling device, providing first void urine with more concentrated human and HPV DNA, is reconfirmed.
- A limitation of this study is that repeated samples from a limited number of subject are analysed. Larger studies are required to demonstrate performance of the assay.

Conclusions:

- These preliminary results confirm that the Papilloplex™ HR-HPV assay is compatible with self-collected first void urine.
- Colli-Pee collected first urine contains higher concentrations of human and HPV DNA compared to urine cup collected first void urine.
- Clinical cut-off determination will be addressed in future studies.

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.

Disclosure:

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