Validation and standardization of the pre-analytical workflow for HPV testing on self-samples and urine samples

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Background:

Cervical cancer global burden

IARC's projections show that unless preventive measures are implemented promptly, the burden of cervical cancer is expected to increase to almost 460 000 deaths per year by 2040,an increase of nearly 50% over the estimated number of deaths in 2018[1].

May 2018 Message «WHO'S Director General's Call to Action to Eliminate Cervical Cancer»[2]

....challenge is to ensure that all girls globally are vaccinated against HPV and that **every woman over 30 is screened** and treated for pre-cancerous lesions.
To achieve this obiective, innovative technologies and strategies are essential . ...

In industrialized countries most cervical cancer cases occur in under screened women [3]

New strategies to improve participation of hard-to-reach women to cervical-cancer screening programs advocate the use of ***self-sampling***

1) https://www.iarc.fr/wp-content/uploads/2019/02/pr264_E.pdf
2) https://www.who.int/reproductivehealth/cervical-cancer-public-health-concern/en/
3) *Verdoodt F, Jentschke M, Hillemanns P, Racey CS, Snijders PJ, Arbyn M. Reaching women who do not participate in the regular cervical cancer screening programme by offering self-sampling kits: a systematic review and meta-analysis of randomised trials. Eur J Cancer. 2015 Nov;51(16):2375-85.*

Background:

New strategies to improve participation of hard-to-reach women to cervical-cancer screening programs advocate the use of ***self-sampling***

Accuracy of human papillomavirus testing on self-collected versus clinician-collected samples: a meta-analysis

Marc Arbyn, Freija Verdoodt, Peter J F Snijders, Viola M J Verhoef, Eero Suonio, Lena Dillner, Silvia Minozzi, Cristina Bellisario, Rita Banzi, Fang-Hui Zhao, Peter Hillemanns, Ahti Anttila

THE LANCET Oncology
Volume 15, Issue 2, February 2014, Pages 172-183

Interpretation In screening programmes using signal-based assays, sampling by a clinician should be recommended. However, HPV testing on a self-sample can be suggested as an additional strategy to reach women not participating in the regular screening programme. Some PCR-based HPV tests could be considered for routine screening after careful piloting assessing feasibility, logistics, population compliance, and costs.

Accuracy of testing self-samples using commercially available **Collection Devices** and **PCR-based HPV assays** needs to be evaluated

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VALHUDES: A protocol for validation of human papillomavirus assays and collection devices for HPV testing on self-samples and urine samples

M. Arbyn[a,*], E. Peeters[a], I. Benoy[b,c,d], D. Vanden Broeck[b,c,d,e], J. Bogers[b,c,d,e], P. De Sutter[f], G. Donders[g,h,i], W. Tjalma[j,k], S. Weyers[l], K. Cuschieri[m], M. Poljak[n], J. Bonde[o], C. Cocuzza[p], F.H. Zhao[q], S. Van Keer[r], A. Vorsters[r]

INTRODUCTION OF *SELF-SAMPLING* SCREENING PROGRAMS REQUIRES:

COLLECTION DEVICES and HPV TEST NEED TO DEMONSTRATE SIMILAR ACCURACY OF RESULTS ON SELF-COLLECTED AS ON CLINICIAN-COLLECTED CERVICAL SAMPLES

CHALLENGES:

A) RISK ASSESSMENT TO AVOID «FALSE NEGATIVE»

1) **ASSAYS' SENSITIVITY CUT-OFF VALUES**

2) **ADEQUACY OF SELF-COLLECTED SAMPLES**

B) VALIDATION REQUIRES STANDARDIZATION OF THE COMPLETE WORKFLOW:
FROM **SAMPLE PROCESSING** TO **NUCLEIC ACID EXTRACTION** TO **HPV DETECTION ASSAY**

LABORATORIES OR SCREENING ORGANIZATIONS NEED TO SELF-VALIDATE, AS NO MANUFACTURER has yet included Self-Sampling INTENDED USE in ASSAY Package Insert.

Lack of standardized protocols for processing Dry Self-collected Swabs for HPV testing is documented by several published studies using different preanalytical sample processing

Authors	Devices	Sample resuspension Volume	Extracted sample Volume	Nucleic Acids Elution Volume	HPV Assay
R. van Baars, (2012) Netherland	Evalyn Brush	**1 mL** PreservCyt®	**250 µl** sample QIAamp MiniElute Virus Spin kit (Qiagen)	**100 µl**	SPF_{10}-PCR system
K. Haguenoer (2014) France	Dry FLOQSwab® Code 529S0C WET FLOQSwab® Code 529S0C	**3 mL** PBS **3 ml** CYMOL	**3 mL** sample was centrifuged, and pellet was used to extract nucleic acids	**????**	INNO LIPA HPV genotyping
F. Sultana (2015) Australia	FLOQSwabs® Code 552C	**4 mL** PreservCyt®	**1 mL** sample was loaded on the Cobas® instrument	**As Per Procedure**	Cobas 4800 HPV Roche
J.K.Jun (2016) Korea	Dry FLOQSwab® 552C	**3 mL** Roche medium	**1 mL** sample was loaded on the Cobas® **1 mL** sample was loaded Abbott 2000 Real-time System	**As Per Procedure**	-Cobas 4800 HPV Roche -Abbott HPV
A. W. Lim (2016) United Kingdom	FLOQSwab® 552C	**3 mL** Roche medium	**1 mL** sample was loaded on the Cobas®	**As Per Procedure**	Cobas 4800 HPV Roche
M. Leinonen (2017) Norway	Evalyn® Brush FLOQSwabs® Code 529S0C	**4.6 mL** PreservCyt®	**1 mL** sample with the Nucli-SENS® easyMag® **1 mL** aliquot was loaded on the Cobas® instrument **1 mL** sample added to the cartridge for Xpert HPV	**50 µL** **As Per Procedure** **As Per Procedure**	- Anyplex II HPV28 (5 µl DNA) -Cobas 4800 HPV Roche -Xpert HPV
F. Modibbo (2017) Nigeria	FLOQSwab® 552C	**3 ml** PBS	**750 µL** samples with the Nucli-SENS® easyMag® (Biomerieux)	**100 µL**	CP5+/6+ LMNX Genotyping HPV assay
K. Decker (2017) Canada	Dry FLOQSwab® 5E046S	**10 mL** SurePath®	**750 µL** samples with the	**As Per Procedure**	Cobas 4800 HPV Roche
M. Saville (2018) Australia	FLOQSwabs® Code 552C	**4 mL** PreservCyt®	**1 mL** sample was loaded on the Cobas® instrument	**As Per Procedure**	Cobas 4800 HPV Roche
M. Viviano (2018) Switzerland	Cotton Swab FLOQSwab® Code 552C	**20 mL** PreservCyt®	**350 µL** sample was loaded on the NIMBUS-IVD	**50 µL**	Seeplex HPV Seegene
J. S. Smith (2018) USA	Viba brush	**10 mL** vial of Scope mouthwash	Hybrid Capture 2 HPV test (QIAGEN Corp,	**As Per Procedure**	Hybrid Capture 2 HPV QIAGEN

Objectives:

Pilot Study to evaluate HPV detection on POC collected Self-vaginal and Urine samples against the GOLD Standard (Clinician-collected cervical samples) by means of a standardized sample processing workflow:

- Determination of self-collected samples' cellularity as a measure of samples' adequacy
- Concordance in HPV detection by means of 2 different HPV assays
- Vaginal Self-collected dry swab – standardization of resuspension volume

CLINICAL STUDY TO EVALUTE SELF-COLLECTION DEVICES AND COMPLETE WORKFLOW FOR HPV-TESTING

MATERIALS & METHODS

100 women attending the Colposcopy Clinic of San Gerardo Hospital, Monza, Italy.

Sample processing:
Laboratory of Clinical Microbiology,
University of Milano-Bicocca

Women with a recent diagnosis of cervical dysplasia referred to colposcopy

- **100 CERVICAL SAMPLES**
  20 mL PreservCyt®
  (GOLD-STANDARD)
- **100 VAGINAL SELF-SAMPLE**
  FLOQSwabs® (COPAN)
  5 mL PreservCyt®
- **URINE**
  100 COLLI-Pee® , (NOVOSANIS):
  20 mL first-void urine

  50 UrISponge™ COPAN):
  3 mL urine

L-Shape Eso-Endo Cervical FLOQSwab ®

Vaginal Self-collection FLOQSwab ®

Colli-Pee®

UriSponge™

- **Nucleic acids extraction** staring from **1 mL of each sample type** by **NucliSENS easyMAG (bioMérieux)** and eluted in **100 microliters for cervical and vaginal samples**; **40 microliters for urine samples**.
- HPV detection was carried out using **AnyplexII™ HPV28 (Seegene)**

Samples's cellularity as a measure of sample adequacy

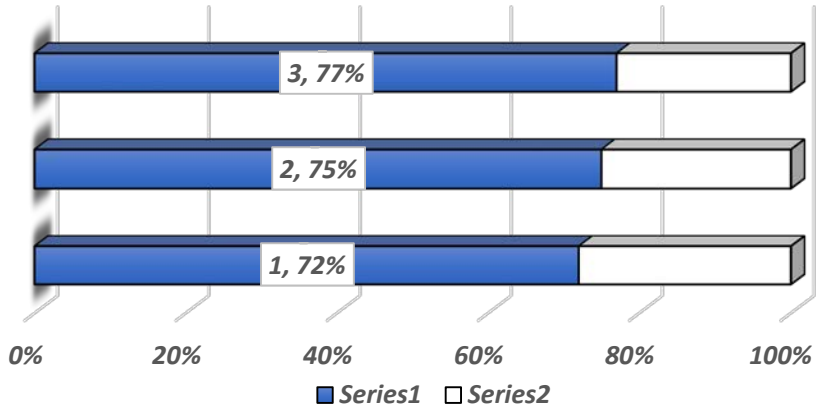
Cellularity	Average (n. cells/sample)	Minimum (n. cells/sample)	Maximum (n. cells/sample)
Cervical Sample (20 ml)	1,71E+06	5,56E+02	2,65E+06
Vaginal Self-Sample (5 ml)	1,38E+06	1,55E+03	1,83E+07
COLLI-PEE (20 ml)	1,81E+06	8,58E+01	3,62E+07
URISPONGE (3 ml)	4,67E+05	2,06E+01	1,34E+07

Sample cellularity was evaluated by means of quantitative real-time PCR detecting human CCR5 gene

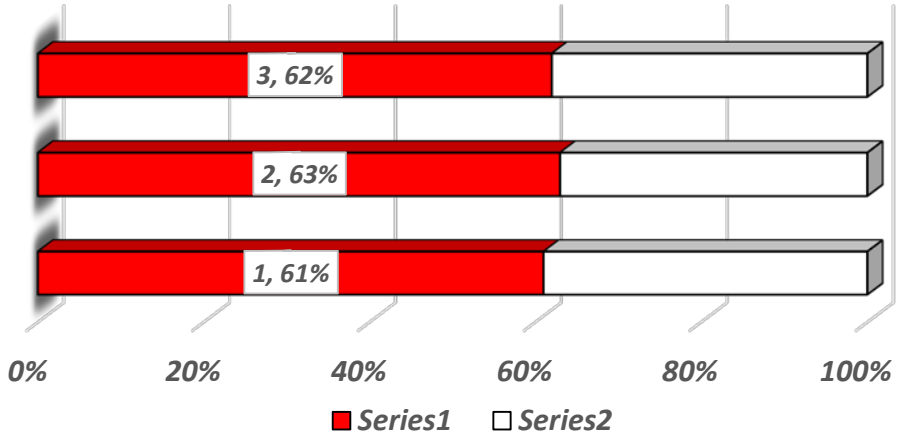
Seegene Anyplex II™ HPV28

HR HPV positivity in **100** Cervical, Self-vaginal and Urines

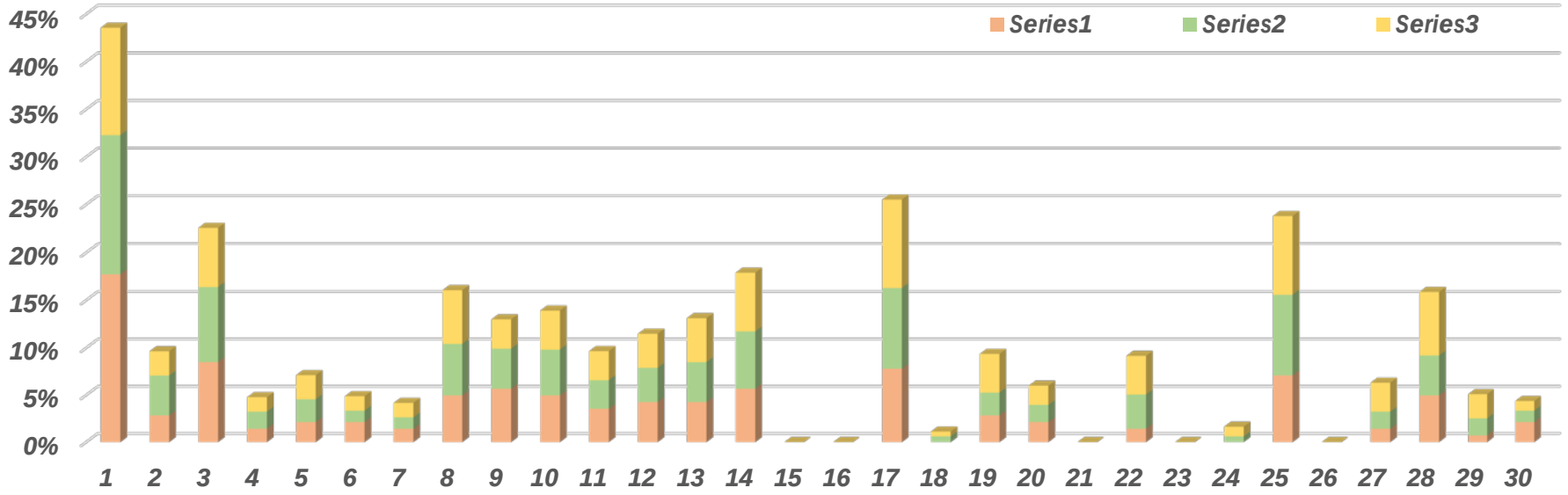
Positivity for one or more HPV types

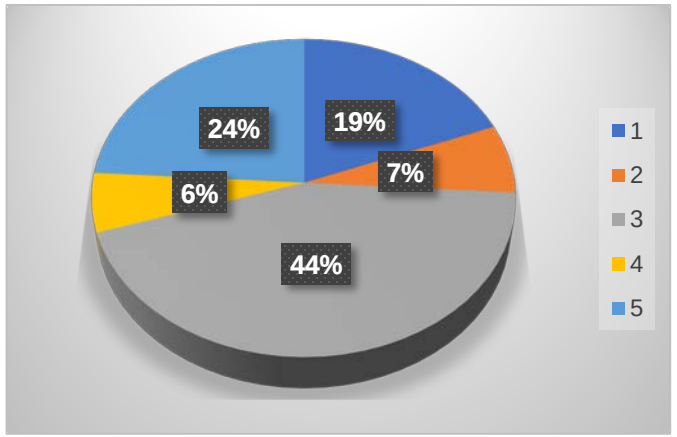


Positivity for one or more high risk HPV types

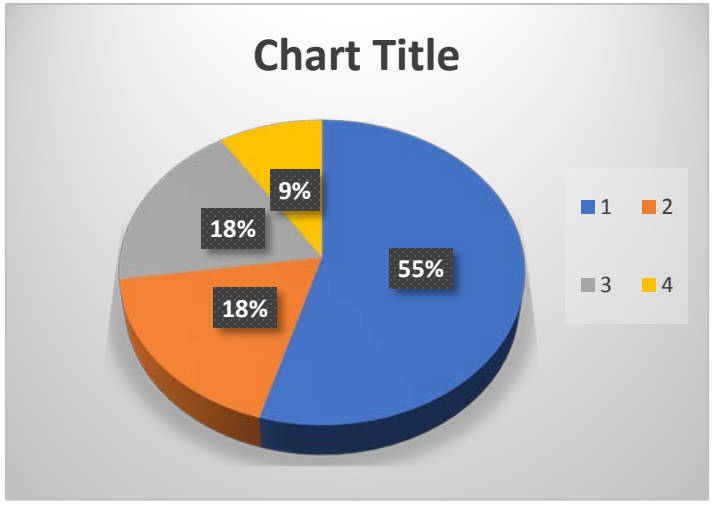


HPV types detected





22 (22%) women



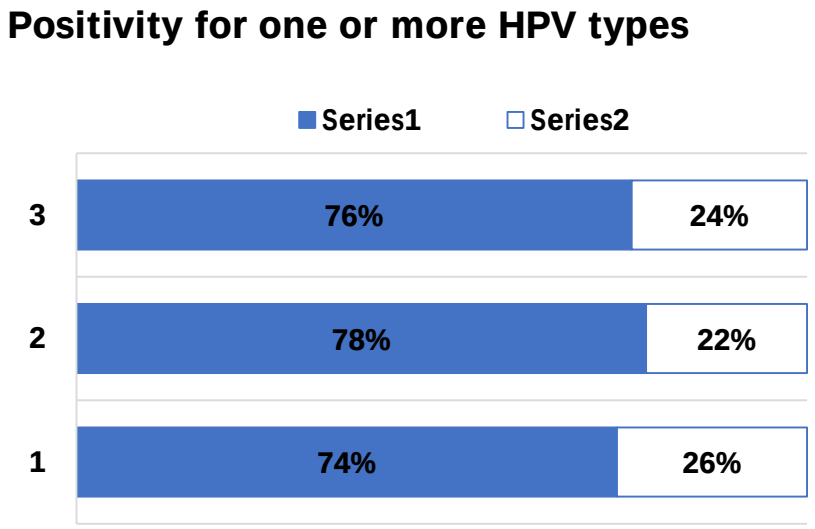
Agreement compared to biopsy

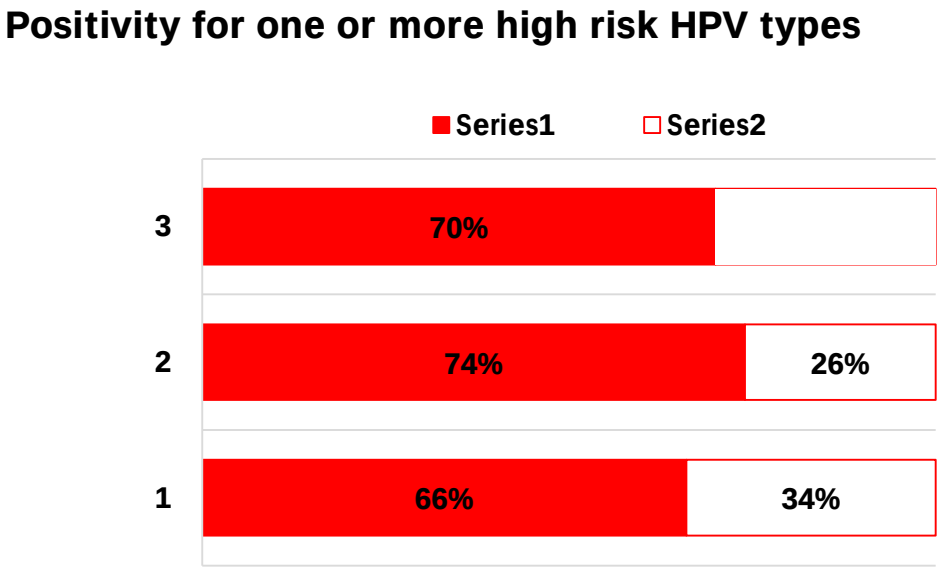
ID	Cytology	Biopsy	Cervical Sample	Vaginal Self-Sample	Urine
M004	HSIL	CIN 3	31	31	31
M008	ASC-H	CIN 3	16, 33, 39	16, 33	16, 33
M024	AGC-US	CIN 3	16	16	16
M031	LSIL	CIN 3	18, 59	18, 59	18, 59
M033	LSIL	CIN 3	16	16	16
M034	ASC-H	CIN 3	31	31	31
M035	HSIL	CIN 3	45, 68	45, 68	45, 68
M040	LSIL	CIN 3	31, 52	31, 52	31, 51, 52
M064	HSIL	CIN 3	16	16	16
M068	HSIL	CIN 3	16	16	16
M071	ASC-US	CIN 3	52	31, 52	52
M083	ASC-H	CIN 3	16	16, 18, 56	16
M002	HSIL	CIN 2	16	16	16, 45
M005	HSIL	CIN 2	52	52	NEG
M012	HSIL	CIN 2	56, 59	56, 59	56, 59
M038	HSIL	CIN 2	NEG	NEG	NEG
M015	LSIL	CIN 1	52, 68	52, 68	68
M027	LSIL	CIN 1	16, 52, 68	16, 52, 68	39, 52, 58, 68
M054	LSIL	CIN 1	66	51, 66	51, 66
M063	LSIL	CIN 1	16	16	16
M020	HSIL	NEG	NEG	NEG	NEG
M022	HSIL	NEG	NEG	NEG	NEG

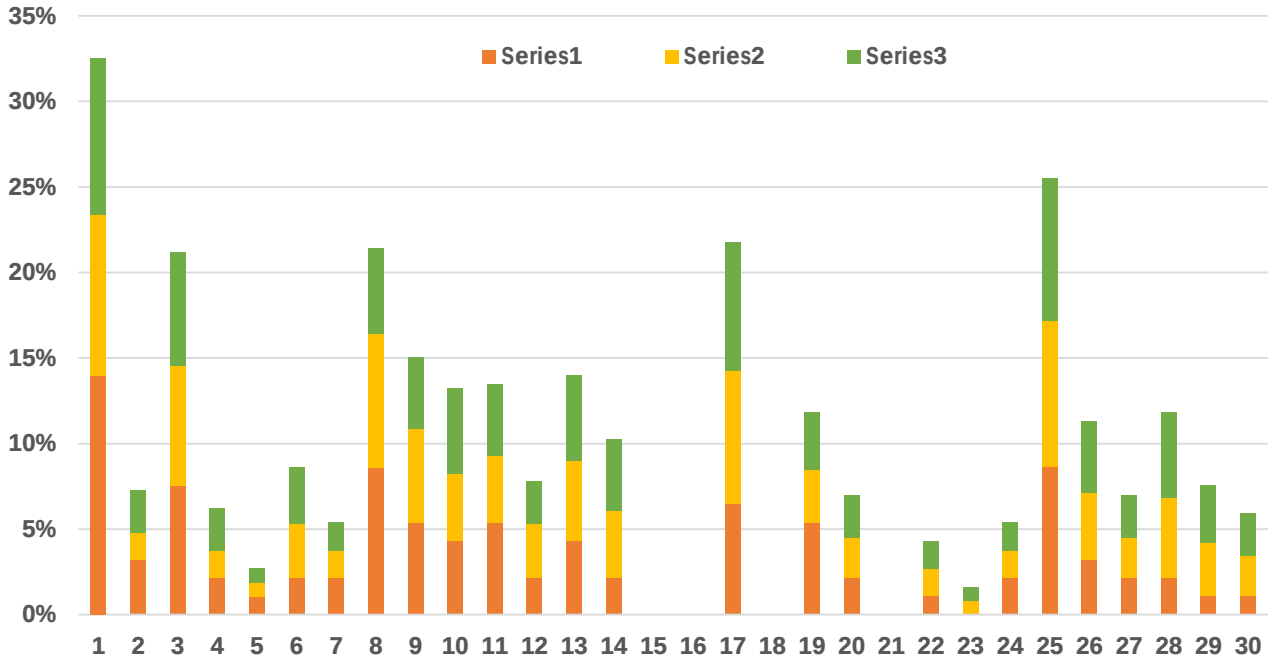
Agreement compared to cervical sample (gold standard)

	Vaginal-self-sample		Urine	
HPV Type	K value (95%CI)	Agreement	K value (95%CI)	Agreement
At least one HR HPV	0.915 (0.834-0.997)	Very good	0.810 (0.692-0.928)	Very good
HPV-16	0.973 (0.920-1.000)	Very good	0.945 (0.870-1.000)	Very good
HPV-18	0.813 (0.406-1.000)	Very good	0.884 (0.658 -1.000)	Very good

HPV detection in 50 urine samples: Colli-Pee ® vs UriSponge™







Agreement compared to cervical sample (gold standard)

	COLLI-Pee®		UriSponge™	
HPV Type	k value (95%CI)	Agreement	k value (95%CI)	Agreement
At least one HR HPV	0.811 (0.636-0.985)	Very good	0.817 (0.645-0.988)	Very good
HPV-16	0.826 (0.636-1.000)	Very good	0.826 (0.636-1.000)	Very good

Pilot Study: Evaluation of Self-Collected samples vs Cervical samples on **BD Onclarity™ HPV Assay on the BD Viper™ LT System**

- 60 women enrolled in the study were evaluated:
  - **60 cervical samples in 20mL of PreservCyt**
  - **60 self-collected vaginal in 5mL PreservCyt**
  - **60 first-void 20mL urine samples**

- Nucleic acid extraction and HR HPV detection was carried out using an automated workflow staring from **0.5 mL of resuspended sample** on
  BD Onclarity™ HPV Assay on the BD Viper™ LT System

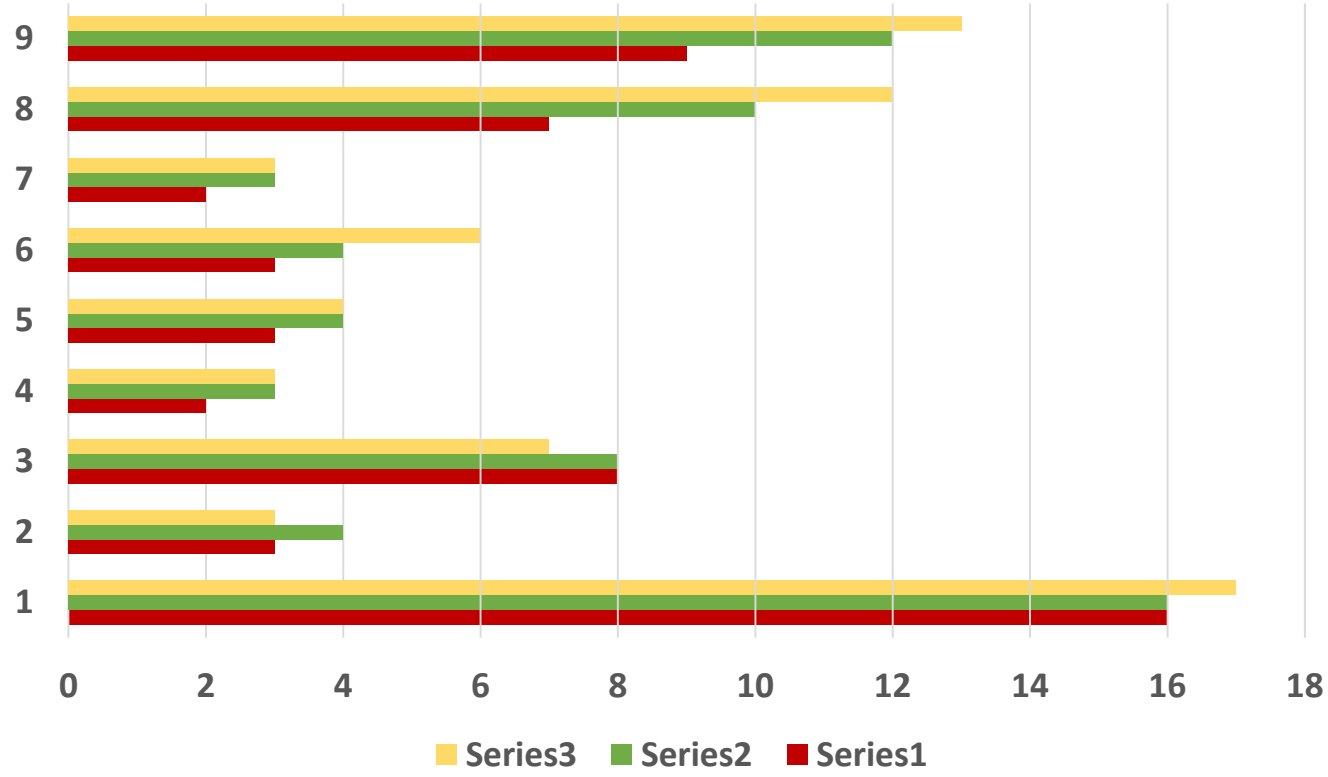
- **BD Onclarity™ HPV Assay** targets E6/E7 DNA oncogenes
  - for 6 discrete HR HPV genotypes:16, 18, 31, 45, 51 and 52
  - remaining 8 HR HPV types reported in 3 groups:

  P1 (33, 58), P2 (56, 59, 66) and P3 (35, 39, 68).

BD Onclarity™ HPV Assay

HR-HPV positivity in 60 Cervical, Self-vaginal and Urine Samples

	Cervical Sample	Vaginal Self-Sample	Urine
HR-HPV positivity	65%	70%	72%



Agreement compared to cervical sample (gold standard)

	Vaginal self-sample		Urine	
HPV Type	k value (95%CI)	Agreement	k value (95%CI)	Agreement
At least one HR-HPV	0.886 (0.762-1.000)	Very good	0.770 (0.598-0.942)	Good
HPV-16	1.000	Perfect	0.875 (0.737-1.000)	Very good

HR HPV genotypes detection above **BD Onclarity™ HPV Assay** "clinical cut-off Ct value"

	N. of sample with at least one result above clinical cut off Ct value	% of sample with at least one result above clinical cut off Ct value
Cervical Sample	**2**	**3.33%**
Vaginal Self-sample	**7**	**11.67%**
Urine	**9**	**15.00%**

Standardization of sample processing prior to nucleic acid extraction

100 Dry Self-collected FLOQSwab Vaginal samples were eluted in 5mL and 20mL of PreservCyt®

	Cervical Sample	Vaginal Self-Sample (5 ml)	Vaginal Self-Sample (20 ml)
Positivity for at least one of 28 HPV	72%	75%	73%
Positivity for at least one HR HPV	61%	63%	61%

	Vaginal Self-Sample (5 ml)		Vaginal Self-Sample (20 ml)	
HPV Type	k value (95%CI)	Agreement	k value (95%CI)	Agreement
At least one HR HPV	0.915 (0.834-0.997)	Very ood	0.874 (0.776-0.972)	Very good
HPV-16	0.973 (0.920-1.000)	Very good	0.945 (0.870-1.000)	Very good
HPV-18	0.813 (0.406-1.000)	Very good	0.813 (0.406-1.000)	Very good

Conclusions

1) Results from this pilot study have shown:

- Self-collected samples have demonstrated adequate cellularity with the devices tested

- HPV testing of self-collected vaginal and urine samples using semi-automated vs automated systems shows good concordance with clinician-collected cervical samples

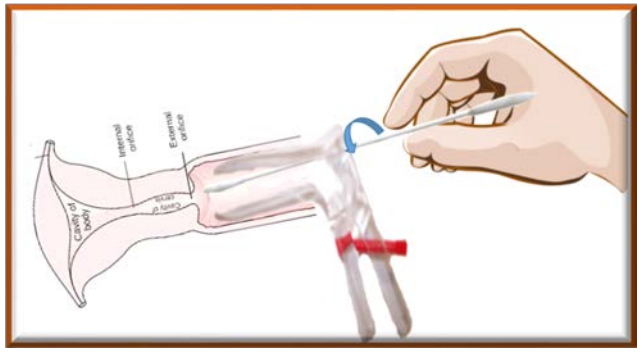
- Need to review sensitivity cut off values of available validated PCR-based HPV assays in order to take into account self-collected sample, collection device and sample resuspension volume.

2) .....European VALHUDES Study due start in the 3rd Quarter 2019 which will include Copan's self-collection devices

Comparison of FLOQSwab®, HerSwab™ and Evalyn®Brush vaginal self collection devices for HPV screening programs

Physician collected Cervical Samples (Gold Standard)

L-Shape Eso-Endo Cervical FLOQSwab®



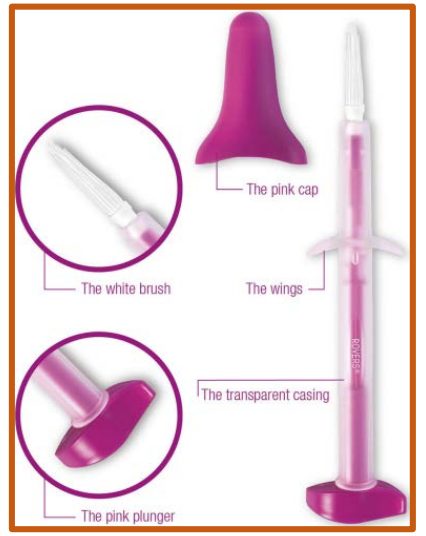
Duplicate samples were collected from patients attending the Colposcopy clinic.

20: **FLOQSwab®** and **HerSwab™**
20: **FLOQSwab®** and **Evalyn®Brush**

Self-collected vaginal samples

FLOQSwab® (Copan)

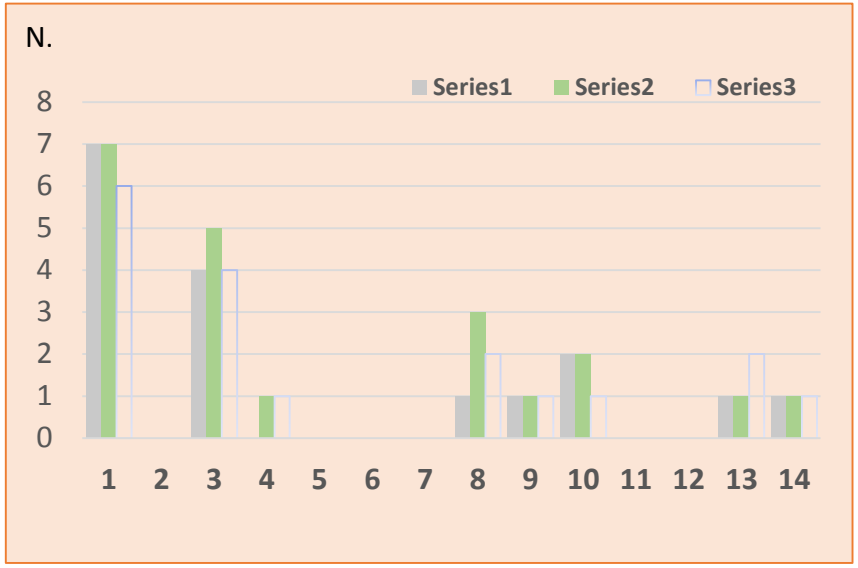
HerSwab™ (Eve Medical)



Evalyn®Brush (Rovers Medical)

RESULTS

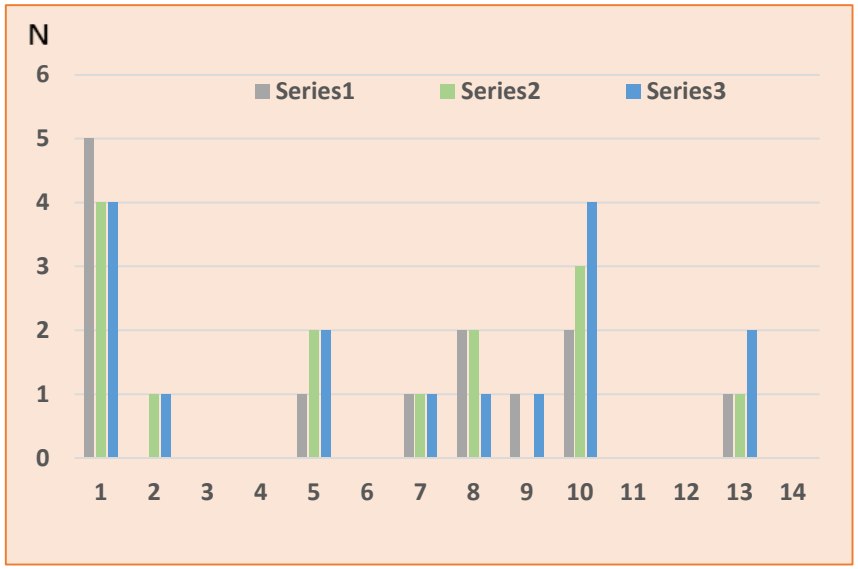
FLOQSwab® vs HerSwab™



	Cervical Sample	Vaginal Self-Sample FLOQSWAB	Vaginal Self-Sample HERSWAB
HR HPV positivity	60%	65%	60%
HR HPV Single Infections	70%	54%	50%
HR HPV Multiple Infections	30%	46%	50%

k= 0.95 Cervical S. vs FLOQSwab®
k= 0.90 Cervical S. vs HerSwab™

FLOQSwab® vs Evalyn®Brush

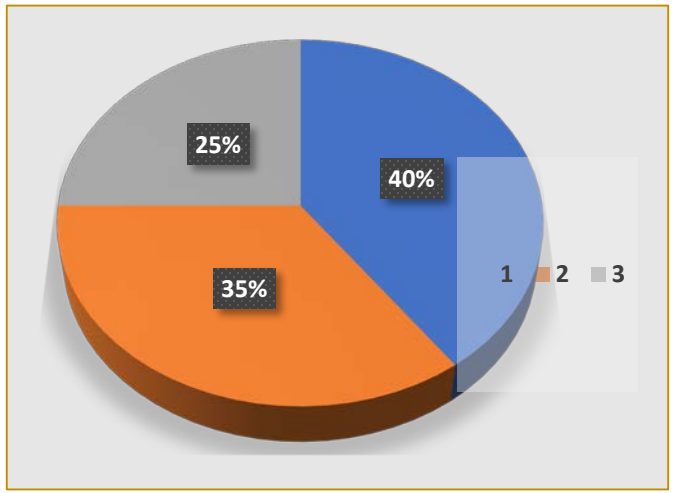


	Cervical Sample	Vaginal Self-Sample FLOQSWAB	Vaginal Self-Sample EVALYNBRUSH
HR HPV positivity	55%	50%	55%
HR HPV Single Infections	82%	70%	64%
HR HPV Multiple Infections	18%	30%	36%

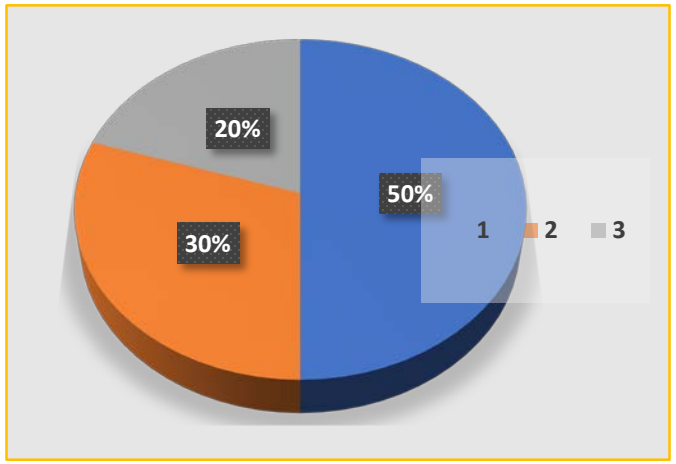
k= 0.95 Cervical S. vs FLOQSwab®
k= 0.81 Cervical S. vs Evalyn®Brush

Comparison of FLOQSwab®, HerSwab™ and Evalyn® Brush vaginal self collection devices for HPV screening programs

FLOQSwab® vs HerSwab™



FLOQSwab® vs Evalyn®Brush



Cost Comparison

	Device cost	Shipping cost
FLOQSwab®	0,60-0,70 €	2.55 €
HerSwab™	7 €	3.55 €
Evalyn®Brush	2 €	2.55 €

Devices	Device cost	Shipping cost
UriSponge™	1.0€	2.55 €
Colli-Pee®	10€	5.55 €