HPV Selfy™ test, an innovative HPV genotyping assay without DNA extraction for vaginal swab self-collection: performance in a referral population of women affected by CIN2+ lesions.

Avian A.[1,2*], Clemente N.[3*], Mauro E.[1], Montrone M.[1], Del Fabro A.[3], Morini S.[3], Perin T.[4], Puzzi L.[1], Isidoro E.[5], Di Napoli M.[5], Zanconati F.[5,10], Giudici F.[5], Gerin D.[6], Riva E.[7], Angeletti S.[7], Ciccozzi M.[7],Castriciano S.[8], Banks L.[9], Canzonieri V.[4,10], Marini B.[1], Sopracordevole F.[3#] and Ippodrino R.[1#].

1) Ulisse BioMed Labs, Area Science Park, SS 14, km 163.5 Trieste (Italy); 2) Molecular Genetics and Biotechnology PhD study programme, University of Nova Gorica (Slovenia); 3) Ginecologia Oncologica, IRCCS - Centro di Riferimento Oncologico (CRO) (Istituto Nazionale Tumori), Aviano (Italy); 4) Anatomia Patologica, IRCCS – CRO (Istituto Nazionale Tumori), Aviano (Italy); 5) Azienda Sanitaria Universitaria Integrata di Trieste, Anatomia Patologica, Trieste (Italy); 6) Azienda Sanitaria Universitaria Integrata di Trieste, Dipartimento di assistenza territoriale, Trieste (Italy); 7) Policlinico Universitario Campus Biomedico, Roma (Italy); 8) Copan Italia Spa, via F. Perotti 10, 25125 Brescia (Italy); 9) International Centre for Genetic Engineering and Biotechnology, Trieste (Italy); 10) Dipartimento di Scienze Mediche, Chirurgiche e della Salute, Università di Trieste (Italy).

*These authors contributed equally to this work.

#These authors both contributed as last authors.

Introduction

Human Papillomavirus (HPV) is considered to be one of the main etiological cause for the development of cervical intraepithelial neoplasia (CIN) and cervical cancer. Despite national cancer screening programs allowed to significantly reduce cervical cancer incidence, participation is still limited mostly by psychological barriers such as emotional distress or fear of pain during pelvic examination. HPV DNA tests coupled with self-sampling devices can increase women participation to the screening. HPV Selfy™ (Ulisse BioMed) is a CE-IVD PCR-based test specifically optimized for self-sampling specimens collected with FLOQSwabs® (Copan, Brescia, Italy); able to directly detect and genotype 14 High-Risk HPVs (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68), through High Resolution Melting (HRM) analysis. The assay is based on an innovative platform (SAGITTA™) for direct nucleic acid detection, without any DNA extraction step, thus shortening overall assay time and saving costs. SAGITTA™ platform is compatible also with other speciemens, such as extracted DNA, cells, Thin Prep™, Formalin Fixed Paraffin-Embedded tissues (data not shown). LadyMed HPV Test™ (Ulisse BioMed) is an innovative service that provides a CE-marked home-collection vaginal swab, a shipping service of the swab to a certified lab hub, HPV detection with HPV Selfy™ technology and a web app for users registration and results delivery on smartphone (Figure 1).

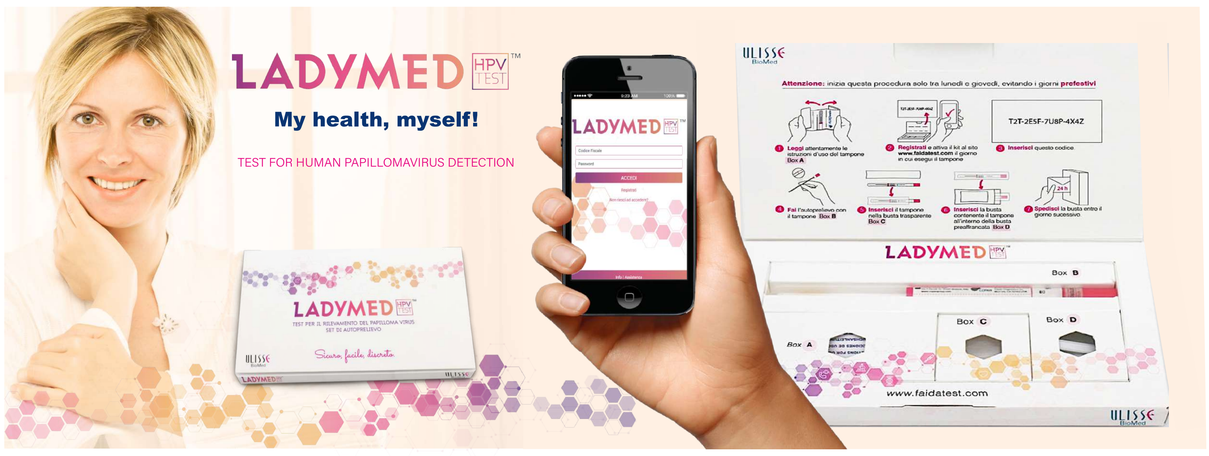

Figure 1. LadyMed: an innovative digital service for HPV testing based on non-invasive self-collection.

Objective

Objective of this study was to validate HPV Selfy™ test in a referral population of women that were histologically diagnosed with CIN lesion at least grade 2 (CIN2+), using the FDA-cleared Hybrid Capture 2 (HC2) High-Risk HPV DNA test (Qiagen, Hilden, Germany) as comparator test. The ongoing clinical study is performed in collaboration with Centro di Riferimento Oncologico, Aviano, Italy (CRO), after Ethical Committee approval (n.17149 30/5/18). According to the international guidelines for HPV test validation (Meijer et al., 2009) in order to achieve a test power of the non-inferiority test greater than 99%, a sample size of 100 women with histologically confirmed CIN2+ lesions should be tested.

Methods

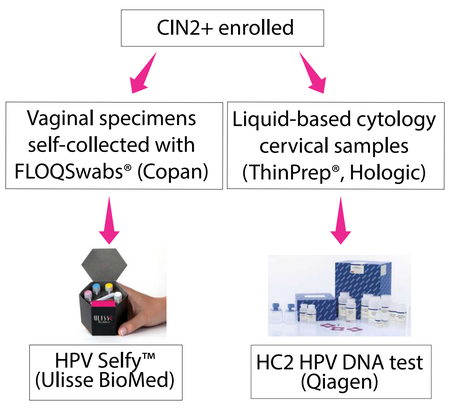

Figure 2. Clinical study workflow.

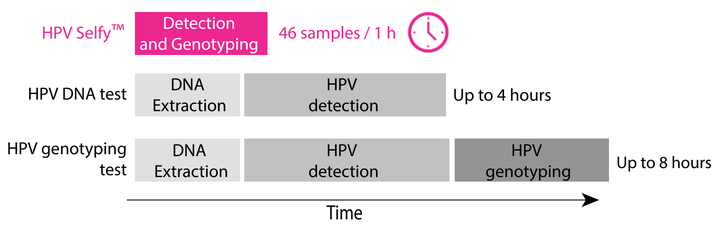

Figure 3. HPV Selfy™ protocol timeline in comparison with other tests.

Women with a histologically confirmed diagnosis of CIN2+ lesion referring to CRO ambulatories for CIN2+ treatment were asked to participate to the study. Only patients who returned the signed informed consent were enrolled in the study. Women received written instructions illustrating the dry FLOQSwabs® self-sampling procedure. Paired self-collected vaginal swabs and clinician-collected cervical brushes were collected from enrolled patients. The study was conducted in accordance with Helsinki Declaration.

Results

Nowadays, 73 patients were enrolled. Among these, 67 samples were considered valid for the analysis. A description of the study population main characteristics is presented in Table 1. Performance of the assay on self-collected samples was compared with HC2 High-Risk HPV DNA test (Table 2). Sensitivity of HPV Selfy™ for the detection of HPV infection was 98% (Table 3). HPV Selfy sensitivity (88.1%) was the same as HC2 (88.1%) in the detection of CIN2+ lesions, showing an excellent overall positive agreement: 0.97 between the two tests (Cohen's Kappa index: 0.858 [95% CI: 0.66 to 1.00]) (Table 4).

Baseline characteristics of the study population (n=73)	
Average age (years)	41.8
25-35 years	26 (36%)
36-45 years	27 (37.5%)
45-55 years	15 (22.4%)
56-65 years	4 (6%)
Smokers (n, %)	24 (32.8%)
Menopause (n, %)	10 (13.7%)
Contraceptive (n, %)	15 (20.5%)
Histological lesions	
CIN2 (n, %)	33 (45.2%)
CIN3 (n, %)	31 (42.4%)
CIN3+ (n, %)	6 (8.2%)
Endocervical adenocarcinoma (n, %)	1 (1.3%)
Microinvasive carcinoma (n, %)	1 (1.3%)
Spinocellular carcinoma (n, %)	1 (1.3%)

Table 1. Description of study population.

HC2 Results	Total	HPV Selfy™ Results	
		Positive	Negative
High-Risk HPV	59	58	1
Negative	8	1	7

Table 2. Comparison between HPV Selfy™and HC2.

HPV Selfy™ vs HC2	Referral population CIN2+ (n=67)
Diagnostic accuracy	0.97 (0.89, 0.99)
Sensitivity	0.98 (0.90, 0.99)
Specificity	0.87 (0.47, 0.99)
Positive predictive value	0.98 (0.90, 0.99)
Negative predictive value	0.87 (0.49, 0.98)

Table 3. HPV Selfy™ performance in comparison with HC2.

	Sensitivity for CIN2+ lesions (n=67)
HPV Selfy™	0.88 (0.78, 0.94)
HC2	0.88 (0.78, 0.94)

Table 4. HPV Selfy™ and HC2 sensitivity for the detection of CIN2+ lesions.

Moreover, to validate the genotyping capability of HPV Selfy™, an additional CE-IVD HPV genotyping test, CLART® HPV2 (Genomica, Madrid, Spain), was used to genotype the HPV-positive samples. CLART® HPV2 Kit detects 14 High-Risk HPVs (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) and 21 Low-Risk HPVs (HPV6, 11, 40, 42, 43, 44, 54, 61, 62, 70, 71, 72, 81, 83, 84, 85, 89, 26, 53, 73 and 82) using a PCR amplification followed by a microarray hybridization assay. To date, results are available for 28 samples, and show high concordance between genotyping capability of the two tests. HPV16 was the most frequently detected genotype (19.4%), followed by HPV31 (8.9%) (Table 5). 7 coinfections of at least 2 High-Risk HPVs were identified by HPV Selfy™ . An example of a coinfection of 2 HPV types (HPV16, HPV56) detected by HPV Selfy™ is reported in the Figure 4.

HPV genotypes	HPV Selfy™ n/frequency(%)	CLART® HPV2 n/frequency(%)
HPV16	13 (19.4%)	14 (20.9%)
HPV18	0	0
HPV31	6 (8.9%)	6 (8.9%)
HPV33	2 (2.9%)	3 (4.5%)
HPV35	2 (2.9%)	3 (4.5%)
HPV39	2 (2.9%)	1 (1.5%)
HPV45	0	0
HPV51	1 (1.5%)	1 (1.5%)
HPV52	2 (2.9%)	2 (2.9%)
HPV56	3 (4.5%)	3 (4.5%)
HPV58	3 (4.5%)	4 (5.9%)
HPV59	0	1 (1.5%)
HPV66	2 (2.9%)	4 (5.9%)
HPV68	1 (1.5%)	0

Table 5. HPV genotypes prevalence.

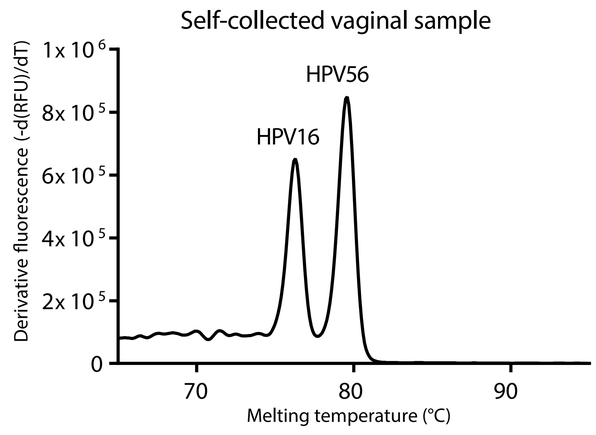

Figure 4. HRM analysis of a sample showing an HPV coinfection (HPV16 and HPV56).

A short questionnaire was distributed to the enrolled population: 91.2% of women reported that self-collection was easy to do and 72% would like to have the possibility to perform self-collection at home (Table 6).

Self-sampling acceptability	Answers
Was the self-sampling easy to do?	91,2% answered YES
Which sampling method was the most uncomfortable? The one performed by clinician or the self-samping?	Sampling by clinician was considered the most uncomfortable by 82,6%
Would you perform self-sampling at home?	72% answered YES
Would you like to have a self-sampling based screening?	61,4% answered YES

Table 6. Questionnaire results.

Figure 5. Home self-collection FLOQSwabs® (Copan).

Conclusions

HPV Selfy™ (Ulisse BioMed) performance was evaluated in a population of 67 CIN2+ patients. The innovative assay showed a substantial agreement with Hybrid Capture 2 (HC2) High-Risk HPV DNA test (Qiagen), suggesting that HPV Selfy™ is a powerful tool for HPV detection and genotyping. The test is directly performed on the samples without DNA extraction, allowing to significantly decrease both time and cost of the whole procedure. Moreover it is capable to provide genotyping information useful for: epidemiological studies, for triage purpose and for vaccine efficacy monitoring. HPV Selfy™ is executed on a common Real Time PCR machine, thus it can be implemented in clinical settings or research laboratories. Furthermore, this study also confirmed high acceptability for self-sampling, which could represent a valuable method to reach under-screened and non responders women in primary cervical cancer screening, independently from age and education level.

References

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a.avian@ulissebiomed.com