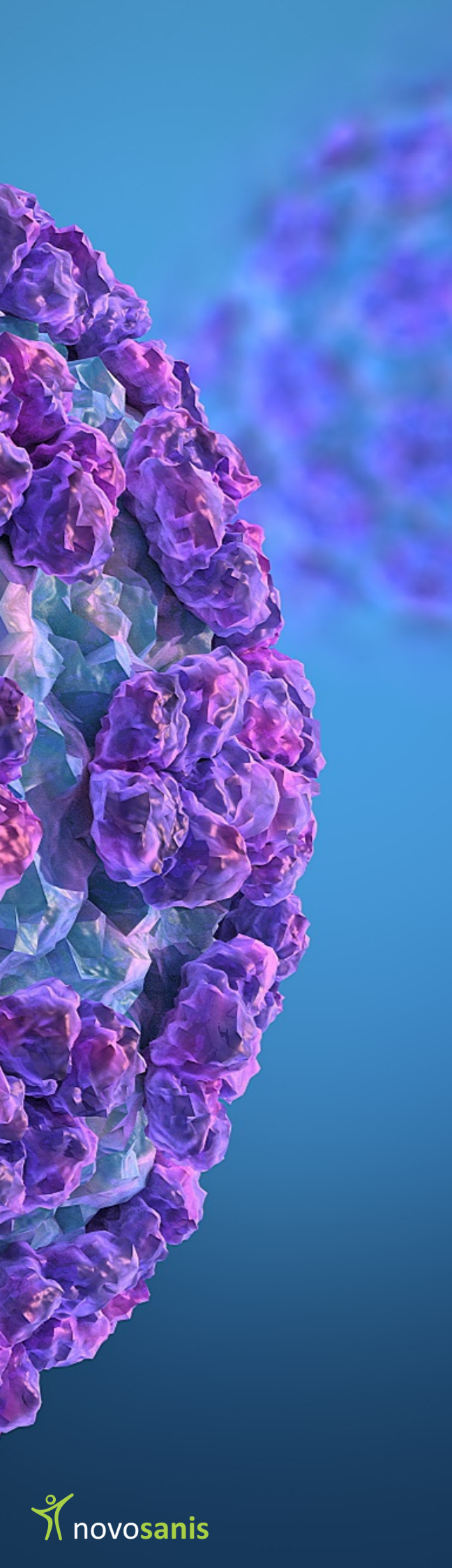


URINE THE 'GOLDEN SAMPLE'

WHY IS URINE SO EXCITING IN
CERVICAL CANCER SCREENING?



“Self-collected urine, through a first-void urine collection device, has the potential to increase uptake of cervical cancer screening. Urine collection can be more comfortable and more socially acceptable for some women who are reluctant to perform a vaginal examination.”

**Professor Clementina Cocuzza,
Department of Medicine and
Surgery, University of Milan-
Bicocca, Italy**

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1. WHAT IS HUMAN PAPILLOMAVIRUS (HPV)?

Human Papillomavirus (HPV) is the most common sexually transmitted infection (STI), which affects the skin and/or lining of cells inside the body. In most people, the infection causes no harm, and disappears naturally overtime. However, persistent HPV infections can lead to long-term damage. Harald zur Hausen, received the Nobel Prize in Physiology or Medicine in 2008 for his discovery on the association between HPV and cervical cancer.

There are over 100 types of HPV, and about 14 high-risk types that are associated with various cancers. HPV16 and 18 are known to be a major cause of cervical cancer in women. High-risk HPV (hrHPV) types are not only limited to causing female cancers. A high percentage of penile cancers have also been linked to HPV16. Certain high-risk groups, such as men who have sex with men (MSM) as well as HIV-positive individuals, are more likely to develop HPV persistent infections. [1]

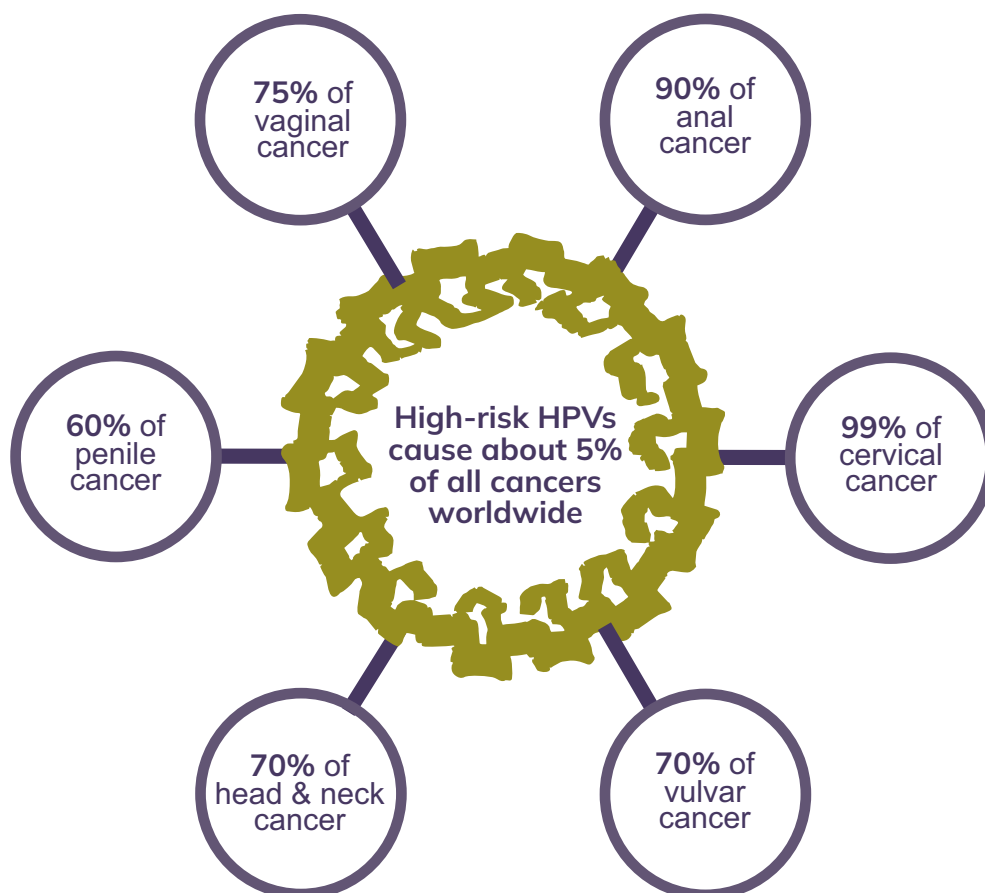


Figure A. Cancers that can be caused by HPV infections

2. HPV VACCINATION: A SAFE AND EFFECTIVE WAY TO PROTECT AGAINST HPV RELATED CANCERS



Protection against HPV infections can reduce the risk of HPV related cancer development.

The HPV vaccine is safe and effective, and is primarily used for young girls, and has recently also been extended to boys aged 12-13 in an increasing number of countries across the globe.

National HPV vaccination programs are implemented in many high-income countries since 2010. Developing countries, such as Bhutan (2010) and Rwanda (2011) were also among the first countries in Asia and Africa respectively to roll-out HPV vaccination programs for school girls [4].

However, in several developing countries, the value of HPV vaccinations is still not well-understood, leading to less uptake of HPV vaccines in these countries. Therefore, vaccine impact studies that demonstrate the potential of vaccination for cancer prevention could help create more awareness and reach a wider group [2].



3. HPV AND CERVICAL CANCER

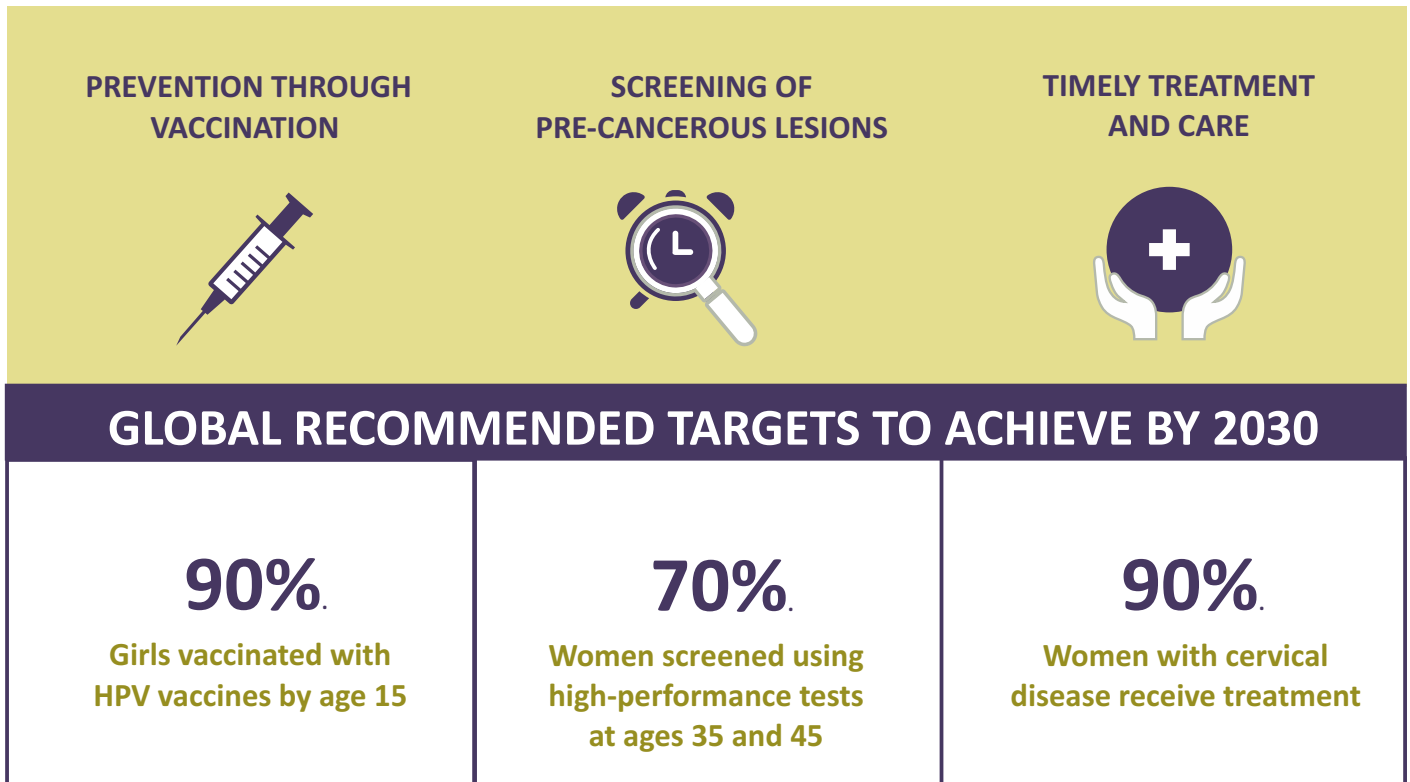


Figure B. The path to eliminate cervical cancer (WHO)

Cervical cancer is the fourth most common cancer worldwide, leading to over 300,000 deaths per year. Most cases of cervical cancer have been linked to types of hrHPV, including HPV strains 16 and 18 [1,2].

To prevent cervical cancer deaths, methods to increase awareness through health education, improving HPV vaccination as well as increasing uptake of cervical cancer screening are needed. The World Health Organization's (WHO) Global Strategy aims to accelerate the elimination of cervical cancer through improved uptake of vaccination and screening as well as timely treatment. Implementation of these methods could prevent more than 40% of new cases and 5 million related deaths by 2050 [3].

4. LIMITATIONS WITH CURRENT CERVICAL CANCER SCREENING METHODS

Early detection of cervical cancer precursors can prevent development of the cancer type. In most countries, regular examinations to monitor healthy individuals are recommended every three to five years for women between the ages of 25 and 65 [5].

Currently two screening methods are used [5]:

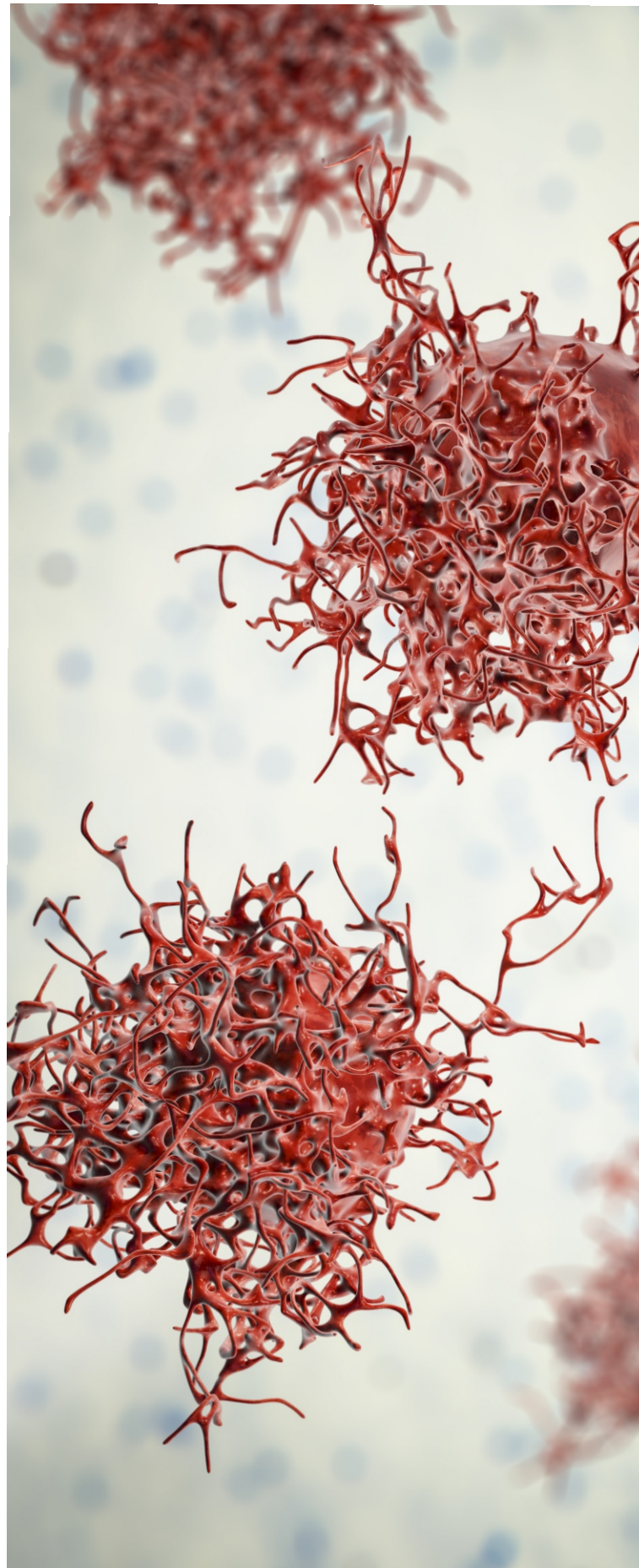
Cytology-based screening: Pap smear

This is the most performed currently used, that involves scraping cells from the cervix, which are examined for abnormalities

HPV-based screening:

Collecting cells from the cervix to check for hrHPV DNA

Screening up-take greatly varies from region to region.



4. LIMITATIONS WITH CURRENT CERVICAL CANCER SCREENING METHODS

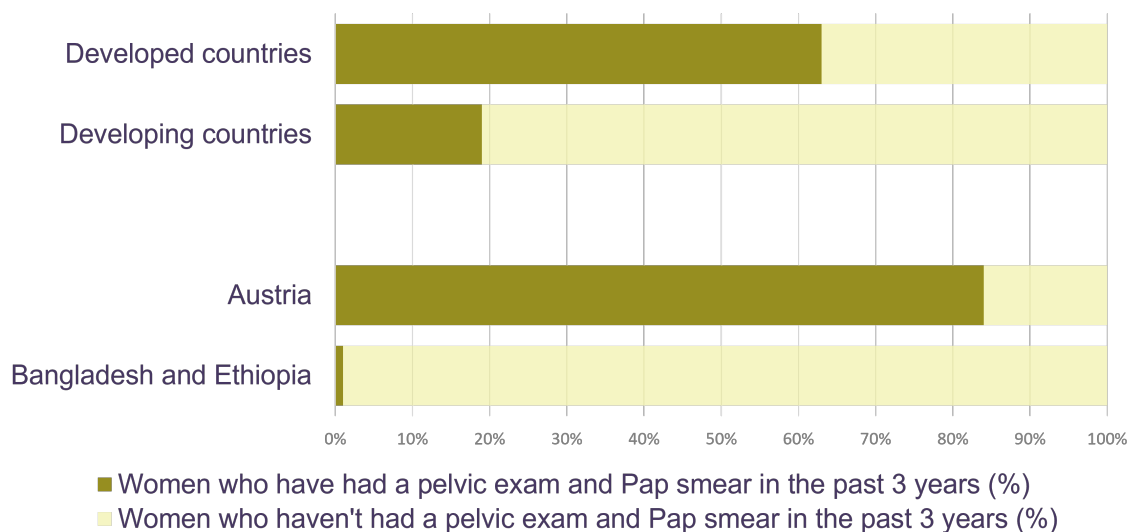


Figure C. Average cervical cancer screening coverage



INVASIVE



COST



**CLINICIAN
DEPENDENT**



TIME-CONSUMING



**SOCIAL & CULTURAL
BARRIERS**

Figure D. Drawbacks of Pap smears

In developed countries, average screening coverage is approximately 63%. Austria and Luxembourg show highest screening participation with about 80% of eligible women reporting a pelvic exam and a Pap smear within the last 3 years. In developing countries, screening rates can drop dramatically, with average screening of 19%. Bangladesh, Ethiopia, and Myanmar show the lowest screening coverage of about 1% or less [6].

Despite the benefits of screening, participation remains low in many areas. Some drawbacks include the methods are invasive, time-consuming and clinician dependent. Additionally, women in some areas are reluctant to have a pelvic examination due to social and cultural barriers [7].

5. URINE SAMPLING FOR HPV DETECTION

A meta-analysis reported an overall 2.14-fold increase in screening coverage due to the use of self-collected samples, highlighting the impact self-sampling methods could have in cervical cancer screening uptake [8].

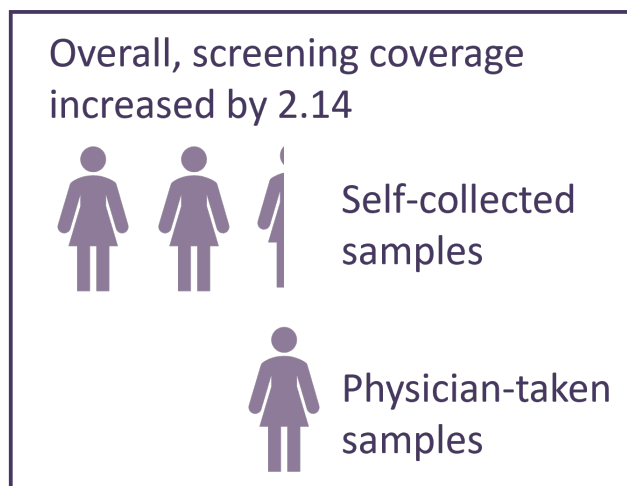
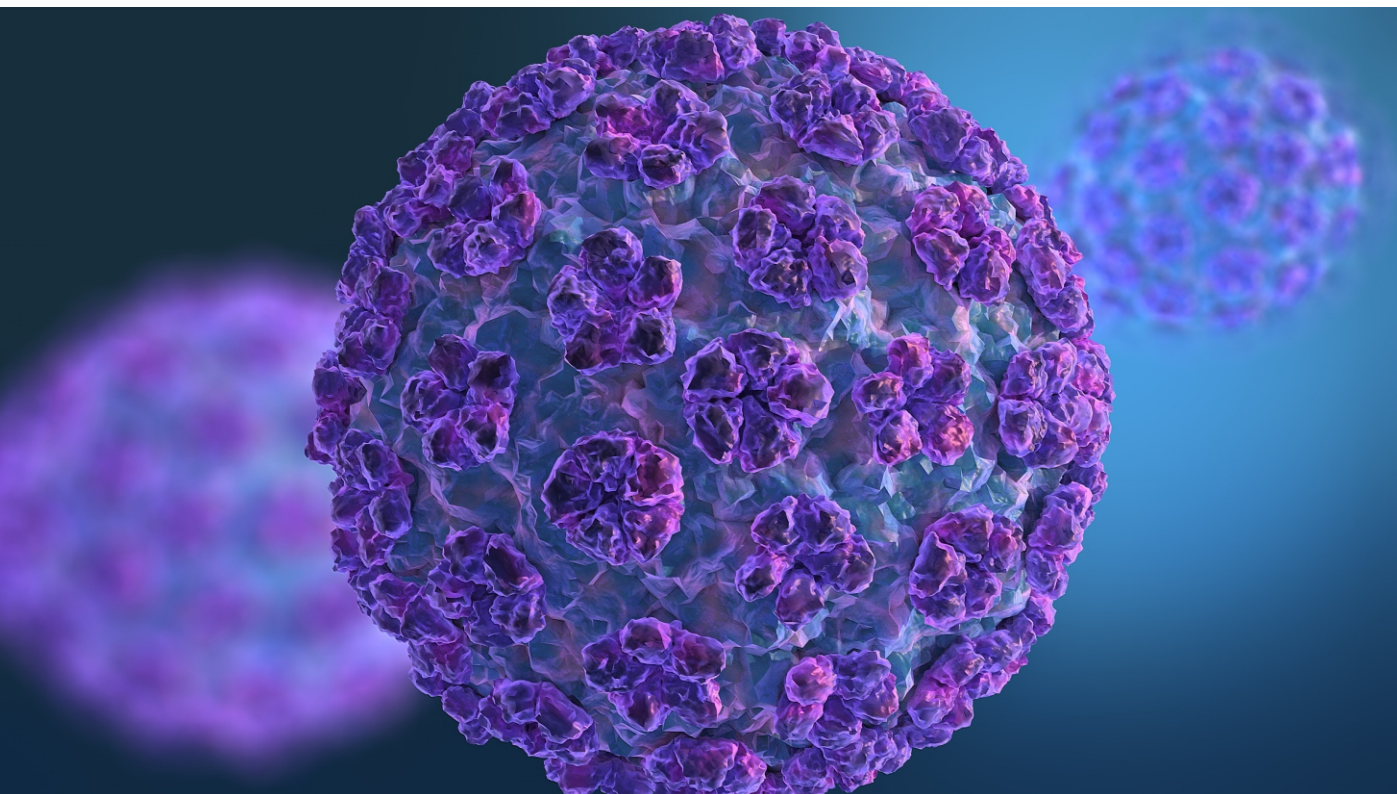


Figure E. Increase in screening coverage due to the use of self-collected samples

Given the challenges women face with a Pap smear, ways to improve screening are required. Self-collection techniques have been recommended, which offer better acceptance.

One method of self-sampling is through urine collection. Women have shown a significantly higher preference to urine as a sample type for hrHPV testing compared to other self-sampling methods [7].



5. URINE SAMPLING FOR HPV DETECTION

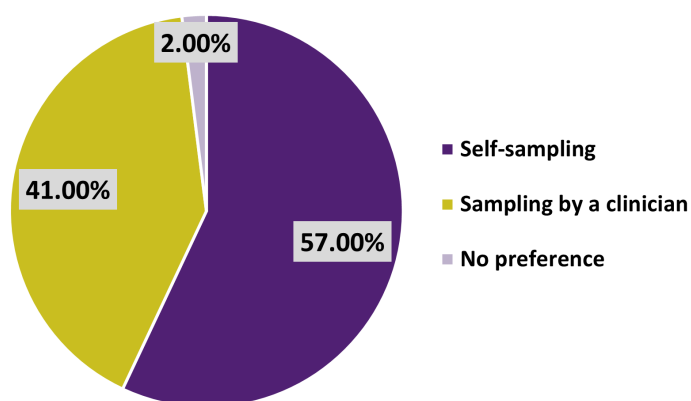


A. Urine: a preferred sample type for HPV detection

Studies consistently report urine for HPV testing as the most accepted and preferred sample type. Urine was chosen in all studies as the most preferred sampling method over vaginal, vulvar, and physician-collected cervical samples for HPV testing to detect high-grade lesions [9]. Women were more confident about providing a urine sample than a vaginal sample for HPV testing and had limited concerns about the accuracy of the test [10].

In accordance with previous studies, the VALHUDES study highlighted that 57% (288/505) of the participants indicated they would prefer self-sample collection at home (urine or vaginal) for their next cervical cancer screening. Among women preferring self-sampling, 52.8% (152/288) indicated their preference for urine collection [11].

What would you prefer at your next screening?



What would you prefer at your next screening?
(For women preferring self-sampling)

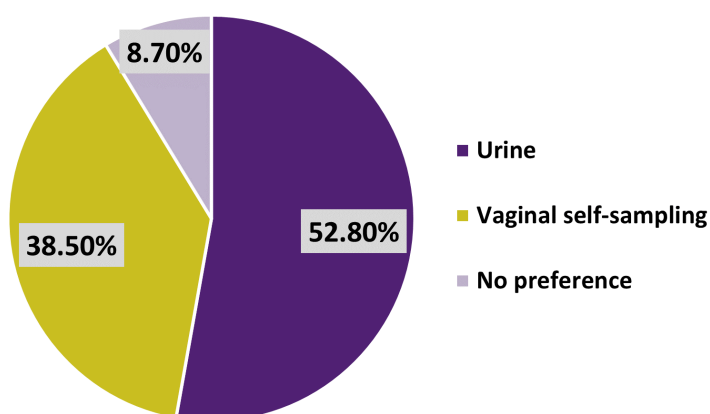


Figure F. Results from the VALHUDES study

5. URINE SAMPLING FOR HPV DETECTION

B. Urine fractions are not the same

However, interestingly not all urine fractions are the same. The epithelial cell layers of the female genital tract, including cell(s) (debris) from lesions induced by HPV, are continuously exfoliated, and washed away into first-void urine (initial 20-30 mL of urine flow). Hence, higher concentrations of HPV DNA are picked up in first-void urine, therefore, collection of this fraction is important to increase sample sensitivity [12].

However, collecting a first-void urine sample with a standard urine cup can be awkward, messy, and inconvenient for the user. Novosanis' Colli-Pee[®], allows for user-friendly urine-collection, as well as volumetric and standardized collection of first-void urine [13]. Moreover, Colli-Pee[®] outperforms a regular urine cup with regards to the number of both human and HPV DNA copies found in urine [14].

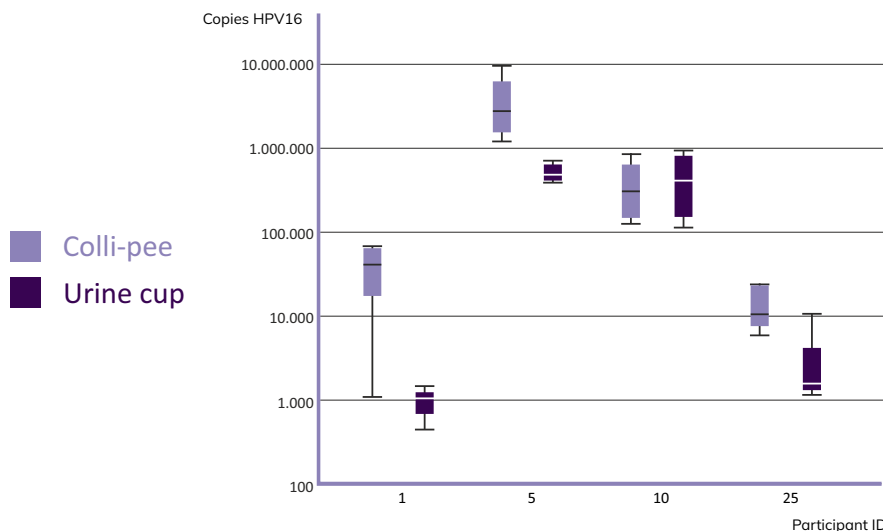


Figure G. Boxplots of HPV 16 DNA copies, for all patients where an infection with HPV16 was detected, found in Colli-Pee[®] versus copies found in a urine cup.



Figure H. Colli-Pee[®] family

The architecture of the Colli-Pee[®] also enables immediate mixing of the sample with a urine preservative, improving the stability of the sample. Colli-Pee[®], pre-filled with non-toxic Novosanis proprietary UCM is recommended for HPV testing. The kit also enables easy postal shipment of the urine sample to the lab for testing [13,15].

5. URINE SAMPLING FOR HPV DETECTION

C. Validation of first-void urine for HPV DNA detection

Several pilot studies confirmed feasibility of HPV DNA detection in first-void urine with commercially available diagnostic assays for automated or POC testing (Roche Cobas HPV, BD Onclarity HPV, Aptima HPV Hologic Panther, Cepheid Xpert HPV) or genotyping (Genefirst Papilloplex HR-HPV, Anyplex II HPV HR Seegene, Fujirebio Innolipa, High+Low Papillomastrip Operon) [16].

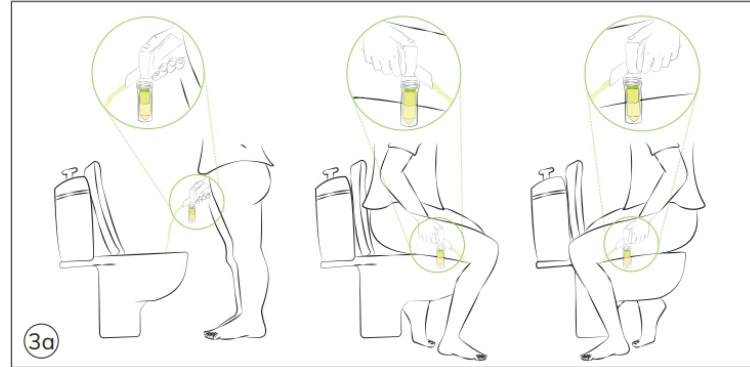


Figure I. Instructions for Use - Colli-Pee® Pee Positions

Clinical trials have been set-up in more than 2500 women referred to colposcopy, to address the performance of Colli-Pee® collected first-void urine to other self-sampling devices for HPV detection and understand its potential in cervical cancer screening [15].

- **EVAH** study showed that first-void urine collected with Colli-Pee® offers very good sensitivity, similar to that of a clinician-taken smear and a vaginal swab self-sample for HPV detection in women with CIN2+ (cervical intraepithelial neoplasia grade 2+) [17].
- **VALHUDES** study aims to assess the sensitivity and specificity of particular hrHPV assays in vaginal self-samples as well as first-void urine compared to matched physician-taken samples. The first results, demonstrate that hrHPV testing with Abbott RealTime HrHPV assay on first-void urine collected at home using Colli-Pee® containing UCM shows similar accuracy for detecting CIN2+ compared to cervical samples taken by a clinician [18].
- **Predictors5.1** study compared five different sampling methods through vaginal self-sampling devices and urine for HPV testing among a population of women referred for colposcopy. Similar positivity rates and sensitivities for CIN2+ and CIN3+ were seen for flocked swab, Dacron swab and urine. Women found urine as a sample easiest to collect and were more confident they had taken the sample correctly using this method [19].

5. URINE SAMPLING FOR HPV DETECTION



D. Cost-effectiveness of self-sampling methods

HPV self-sampling offers opportunities to reach many more women for cervical cancer screening in low- and medium-income countries, where the infrastructure for cervical cytology-based screening is lacking or where there are social and psychological reasons not to participate. Additionally, urine-based HPV testing can be cheaper than standard cytology-based cervical testing as it does not require a practitioner or specialized equipment. Colli-Pee® Small Volumes offers an additional benefit, as its collector tubes are designed to fit many high-throughput laboratory systems, eliminating the need for an additional pipetting step and reducing manual handling errors [7].



Figure J. Colli-Pee® Small Volumes

5. URINE SAMPLING FOR HPV DETECTION

E. Molecular-based triage on hrHPV-positive women to detect cervical cancer

Most hrHPV-positive women have transient infections, which do not result in any clinical symptoms. Only a minority of hrHPV-positive women (20%) harbor a transforming infection, leading to the development of clinically relevant CIN for cervical cancer.

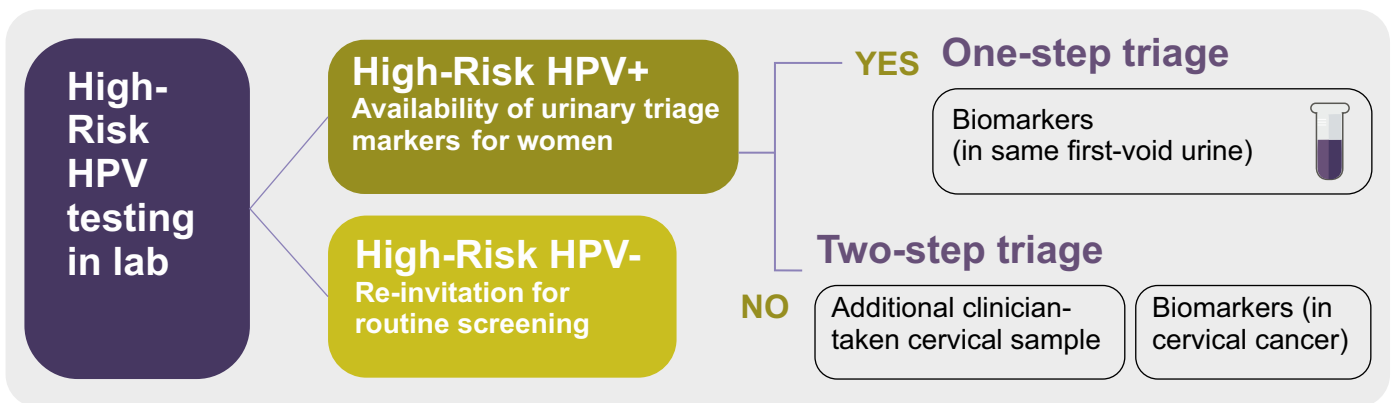


Figure K. HPV triage

Therefore, referral of all hrHPV-positive women to a gynecologist for colposcopy would be unnecessary, leading to overdiagnosis and overtreatment, adding to unnecessary costs and higher risks of obstetric complications. To reduce overdiagnosis and overtreatment of clinically non relevant HPV infections, screening programs must incorporate triage before colposcopy referral.

Detection of molecular biomarkers on hrHPV-positive women can be used for triage to distinguish between clinically relevant, transformative hrHPV infections from transient infections, which will not result in any clinical symptoms and will clear spontaneously.

Many studies have found a strong association between the methylation of host and viral genome with the development of CIN2, CIN3, and cancer. In addition, methylation levels have also been found to rise in parallel with increased severity and duration of disease.

First-void urine sampling also offers the ability to test both primary hrHPV and other molecular biomarkers in the same sample, avoiding repeated sample collection, reducing efforts and costs associated with cervical cancer screening [16].

5. URINE SAMPLING FOR HPV DETECTION

F. First-void urine to monitor HPV vaccine impact

As HPV can be detected in urine, the sample type can also be used to monitor HPV vaccine impact and effectiveness. Researchers carried out studies in Bhutan and Rwanda to monitor the impact of HPV vaccination in schoolgirls. Participants self-collected a urine sample using Colli-Pee®. In both Bhutan and Rwanda HPV6/11/16/18 prevalence was lower in vaccinated than in unvaccinated participants, highlighting the importance of vaccination.

Urine to monitor the impact of HPV vaccination is adaptable to a wide range of settings and populations, making this approach particularly valuable to periodically assess the early impact of HPV vaccination [2].

“I am convinced that the clinical and diagnostic information present in a first-void urine sample still is greatly underestimated. We confirmed that first-void urine is an interesting sample to monitor HPV vaccination programs; possibilities in cervical cancer screening programs are being explored. The non-invasive character of urine sampling, with option of home collection, will definitely help to enroll underserved women in cervical cancer screening and follow-up programs across the world.”

Professor Alex Vorsters, VAXINFECTIO, University of Antwerp, Belgium

6. HIV AND CERVICAL CANCER

Women with compromised immune conditions such as Human immunodeficiency virus (HIV), are more likely to develop persistent HPV infections, leading to higher risk of cervical cancer.

Given the synergy between the two infections, it is critical that women with HIV are screened regularly for HPV.

Further, HIV rates in adolescent girls and young women are particularly high in areas where cervical cancer screening is significantly low. Using urine as a sample type to detect HPV detection could help monitor infections and screen for cervical cancer in HIV-positive women [20].

Women living with **HIV** have a **SIX-FOLD** increased risk of cervical cancer when compared to women without HIV.



Figure L. Invasive cervical cancer is an AIDS-defining illness

So why can urine be considered the ‘golden sample’?

In this e-book, we highlight reasons why urine is such an exciting sample type for HPV-based cervical cancer screening and vaccination follow-up:

- Women prefer urine as a sample type for hrHPV detection, as the method is non-invasive, user-friendly, and suitable for home-collection.
- Self-collection techniques can improve screening uptake. Urine-based screening has the potential to increase participation, and especially reach those women who do not traditionally undergo screening.
- Urine shows similar sensitivities and performance to other sampling methods for hrHPV detection.

However, to use urine for HPV detection for cervical cancer screening, the sample collection process must be optimized through standardized collection of first-void urine and effective preservation. Colli-Pee® offers great potential in this regard.

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