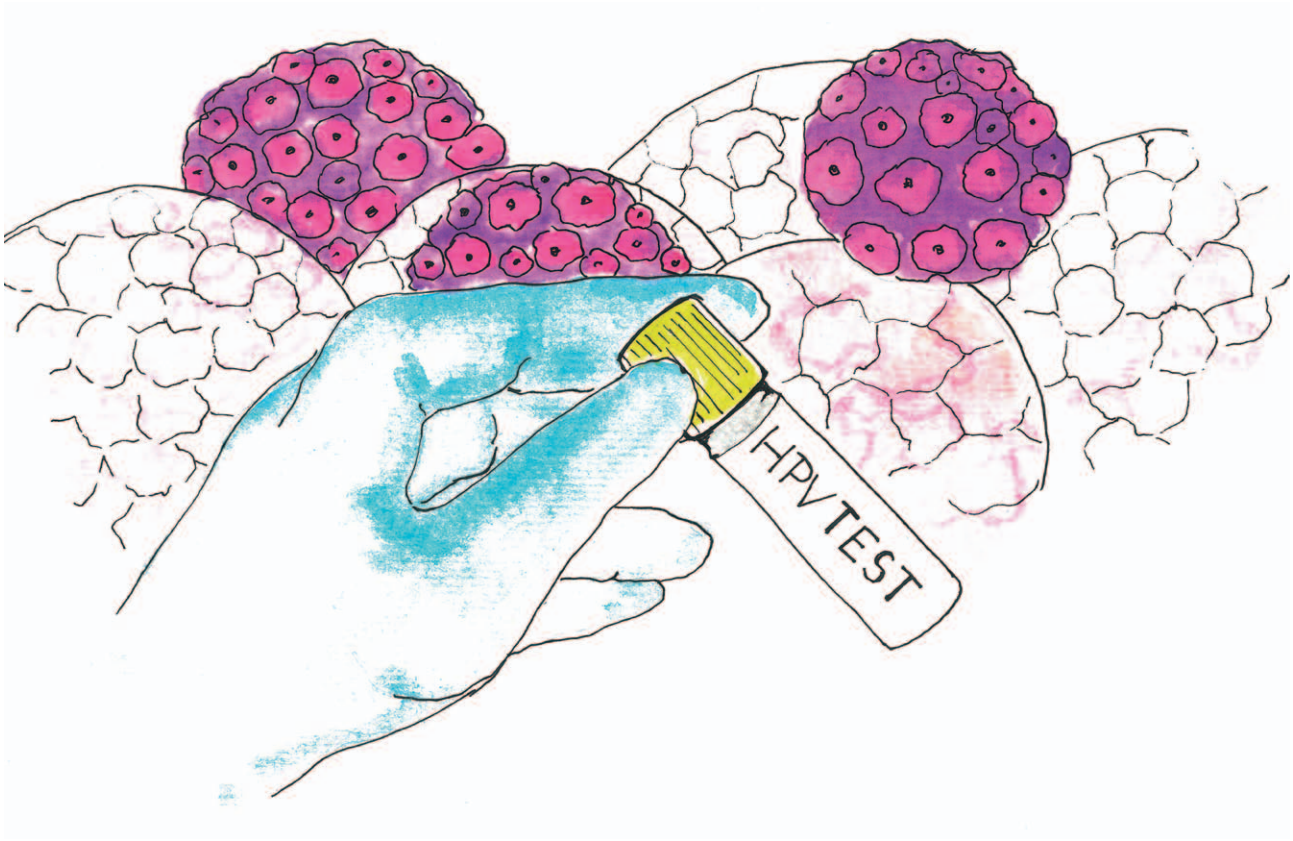
Everything you need to know about HPV tests

Most cervical cancer cases can be prevented by screening and vaccination!



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Contents

What is HPV?

The human papillomavirus, abbreviated HPV, is the most common sexually transmitted infection.

More than 200 types have been identified, and we can divide these into high- or low-risk types. When any of the high-risk types have been in the body for a long time – up to 15 years – they can cause pre-cancerous lesions and even cervical cancer.

HPV is spread primarily but not exclusively through sexual intercourse and can affect both males and females.
Accordingly, HPV is responsible for a considerable amount of genital, anal, and oropharyngeal cancers in both sexes.

HPV is not a stigma – it is a risk!
HPV does not automatically equal cancer!
Cancer is a rare but dangerous complication of HPV.

The role of HPV in pre-cancerous status and in cervical cancer

Knowing more about HPV is essential for all of us. Early detection of infection may give us an opportunity to prevent cancer. When we get infected with HPV, we usually don't notice. It is almost always asymptomatic.

There are two types of epithelium in the cervix (the gateway to the vagina), squamous and glandular. The place where these two epitheliums meet is called the transformation zone, and it is the spot the HPV virus prefers. There is a 'fight' on the line between the two epitheliums, and the virus exploits this. It settles here.

There are two types of HPV infections:

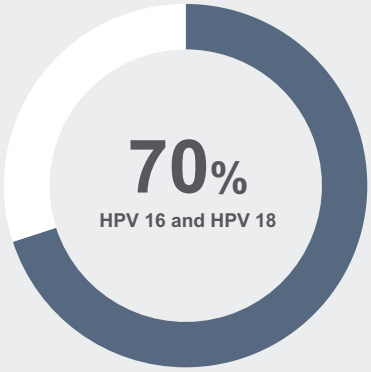
- **Transient** – the viral infection is temporary
- **Transformer** – the infection can be tumour-forming

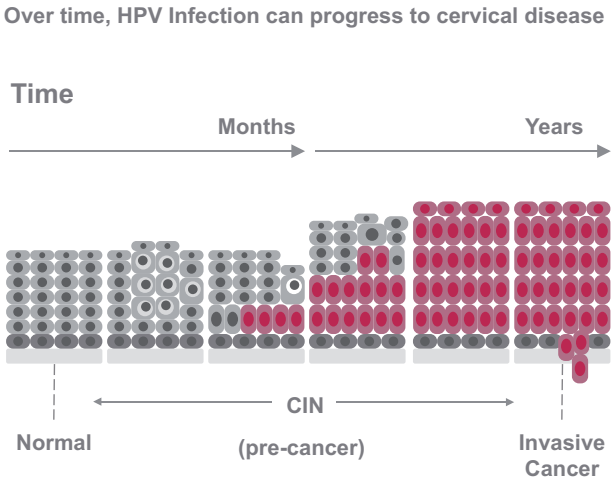
The immune system usually fights against the virus; however, in some cases the immune system cannot get rid of HPV and the body does not yet manifest serious warning signs.

The virus may enter the body locally without entering into the bloodstream. Our immune systems may, in some cases, get rid of the infection, but protection doesn't last long. We can get infected multiple times by the same virus.

When the virus lingers in the body, it transforms its cells, and over the course of years, it may create a pre-cancerous condition. In this stage, the proliferation of abnormal cells has already begun, but it is not yet cancer (transformer infection).

Of the 150+ types of Human Papillomavirus (HPV): HPV genotypes 16 and 18 cause ~70% of cervical cancer cases





Screening programmes can notice these lesions before progression into cancer. With a small intervention called conisation (they will cut out a cone shape, thus cutting out the infection as well), these pre-invasive lesions can be solved in this early stage.

Because the infection has no symptoms, without periodic screening programmes these transforming infections cannot be detected and may eventually lead to cancer.

Cervical cancer can be prevented with HPV vaccination and screenings

The importance of the HPV vaccine

The role of the HPV vaccine is to teach the immune system to prevent primary infections before it is exposed to HPV for the first time. This is the primary prevention of cervical cancer.

For more information about the HPV vaccine read the ENGAGe HPV vaccine brochure.

The importance of screenings

The task of screenings is to find the virus or pre-invasive early lesions soon so that there is no need for intervention or, if an intervention is necessary, to keep this treatment as minimal as possible. Screening services and treatment for pre-cancerous lesions are important secondary prevention.

Screening types

Why are we talking about screening types in plural?

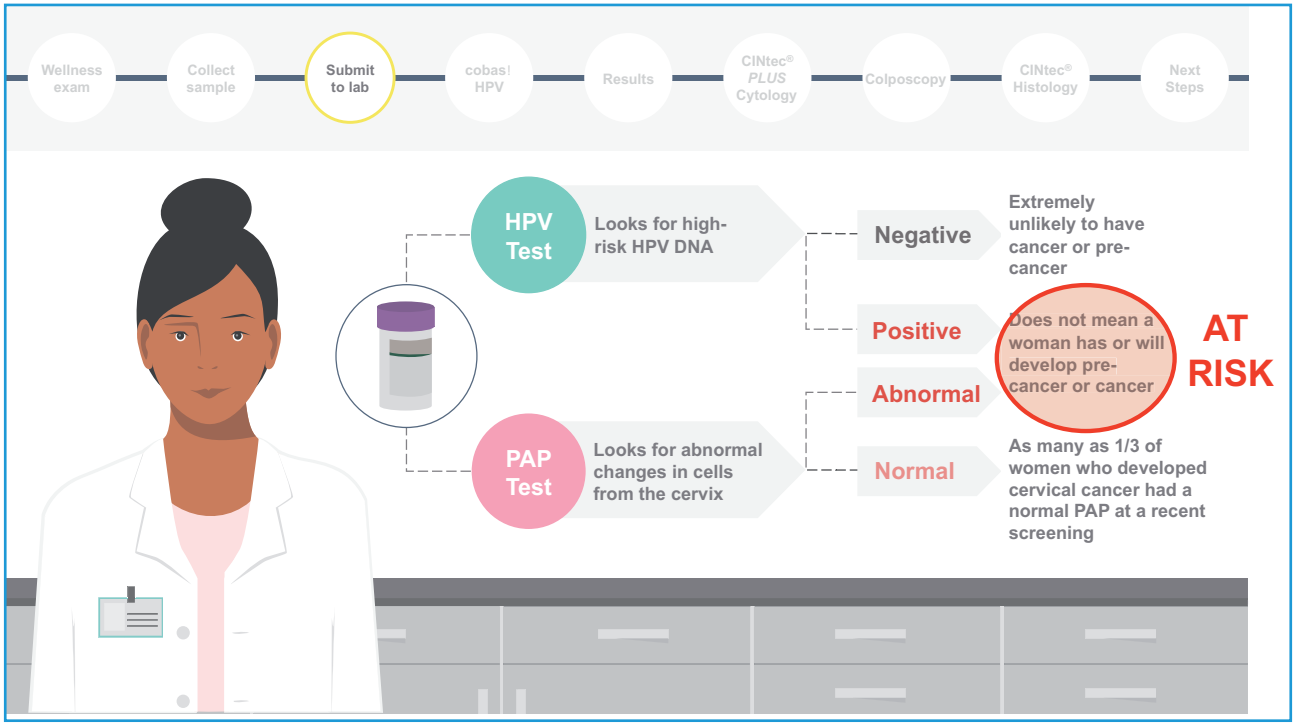
Because we have 3 types of screening:

1. Cytology – PAP smear test: Its job is to show if there are any lesions in the cervix. A lesion could be an inflammation, a problem caused by bacteria or fungus, or something more serious. A brush is used to sample the cervix and the cells sampled are evaluated in a laboratory by pathologists or cyto-technicians. If abnormal cells are detected in a Pap smear test, a biopsy is needed to confirm it. A cancer diagnosis cannot be made at this stage.

2. HPV test: It shows if there is human papillomavirus in the cervix. Various companies offer several types of HPV tests. Some indicate whether there is an infection or not (high-risk HPV positive or not), while others detect the two most important high-risk types (HPV 16, 18) and others can detect all HPV types.

None of those tests can confirm whether you have cancer or not.

3. HPV self-sampling: This test can be performed at home individually and then sent directly to a laboratory. The results of this test come to the patient, who contacts her gynaecologist if it is positive. Within this category, more research is ongoing, and in the future some urine or blood tests may also be available.



Unfortunately, many women don't go for screening and are thus increasing their risk of not detecting cancer.

On the other hand, it may happen that someone goes for screening regularly, receives negative results, and still develops cancer. This happens rarely but does occur. There may be several reasons: test reliability, quality, sensitivity; menstrual cycle timing; height of the transformation zone; how much mucus the smear samples; and the person who takes the sample and analyses the results. These reasons are especially true for cytology-smear tests, resulting in low sensitivity (detection) rates for cancer. On the other hand, HPV tests are DNA-based tests with lower false-negative rates and can detect more cancers by screening.

The scientific concepts of sensitivity and specificity explains how this can be true.

- Specificity: the probability that the value of the diagnostic test will be negative in a patient who does not have the disease being tested. Specificity thus characterises how reliably the test identifies those in whom the parameter under study is not abnormal.

It does not give a positive result for a sample that is truly negative.

- Sensitivity: the probability that the diagnostic test will be positive in a patient with the disease. Sensitivity characterises how reliably a test detects the presence of a disease.

A positive test shows with certainty that someone has a lesion or cancer.

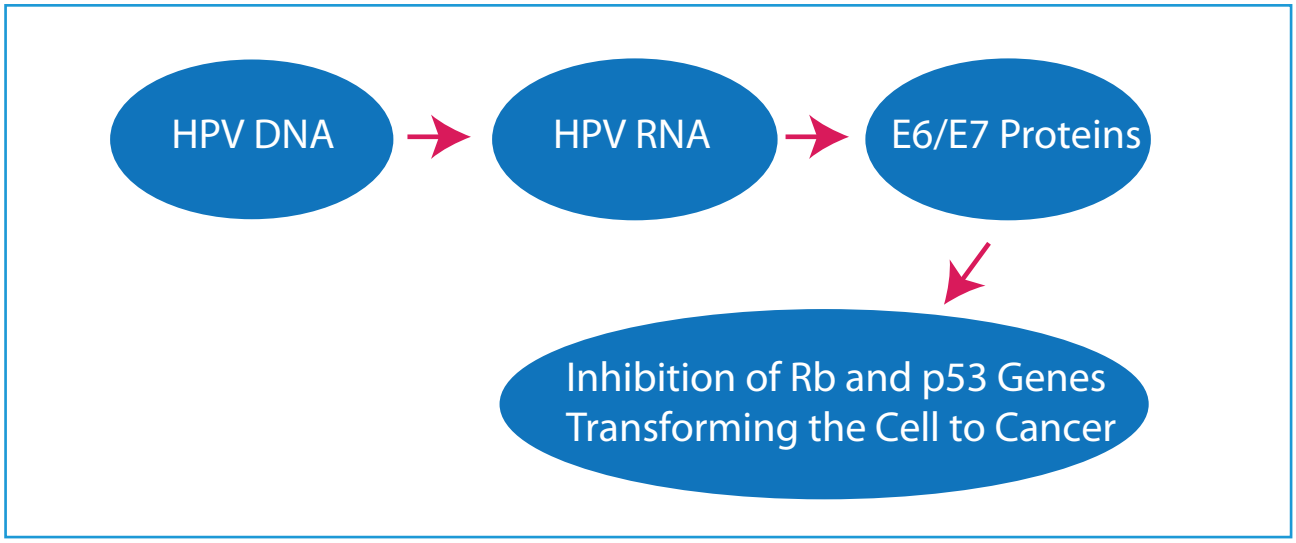
Tests may vary in sensitivity and specificity.

The cytology test has approximately 60–70% sensitivity. This means that in a smear test, 30–40% of the condition is not detected. At the same time, this procedure is by no means superfluous, as it can screen out abnormal processes in approximately 70% of cases.

In contrast, the sensitivity of the HPV test is over 90% in detecting high-risk HPV infection and pre-invasive diseases. **(1)**

HPV tests

HPV tests detect the genetic material of the virus (DNA or RNA). When HPV is involved in transforming infections, at first, HPV DNA is produced in large amounts. From this comes RNA. RNA then produces E6–E7 proteins, which are important in cancer development. To sum up, the virus first produces DNA, then RNA, and finally the E6–E7 proteins.



Strong evidence supports that screening using tests that detect the nucleic acids of oncogenic HPV types is more effective, in terms of reducing the incidence and mortality from this cancer, than screening with cytology **(2-4)**

This doesn't mean that cytology is not useful, but implementing HPV tests first is the best way. If the HPV test shows positive results, then the cytology needs to be clarified further. That's because if the HPV test is positive, we do not know if a risky a cervical lesion is also present – the test may indicate a simple transient HPV infection. In order to prevent unnecessary referral to gynaecologists, a PAP smear can be used to triage those patients who are already shown to be HPV-positive. To clearly see what the next step is, we need the cytology to show if a lesion is present. (CIN1, CIN2, CIN2, HSIL, LSIL, ASCUS, AGC-NOS)

The age at which it is recommended to start HPV testing varies by country, from 30 or 35 years of age. Depending on the local cancer numbers in your country, screening may be started as early as age 25. This is because HPV infections are more common under the age of 30, and they are usually the transient type. Over the age of 30, in most cases, the infection is transformative, so there is a higher chance of developing cancer.

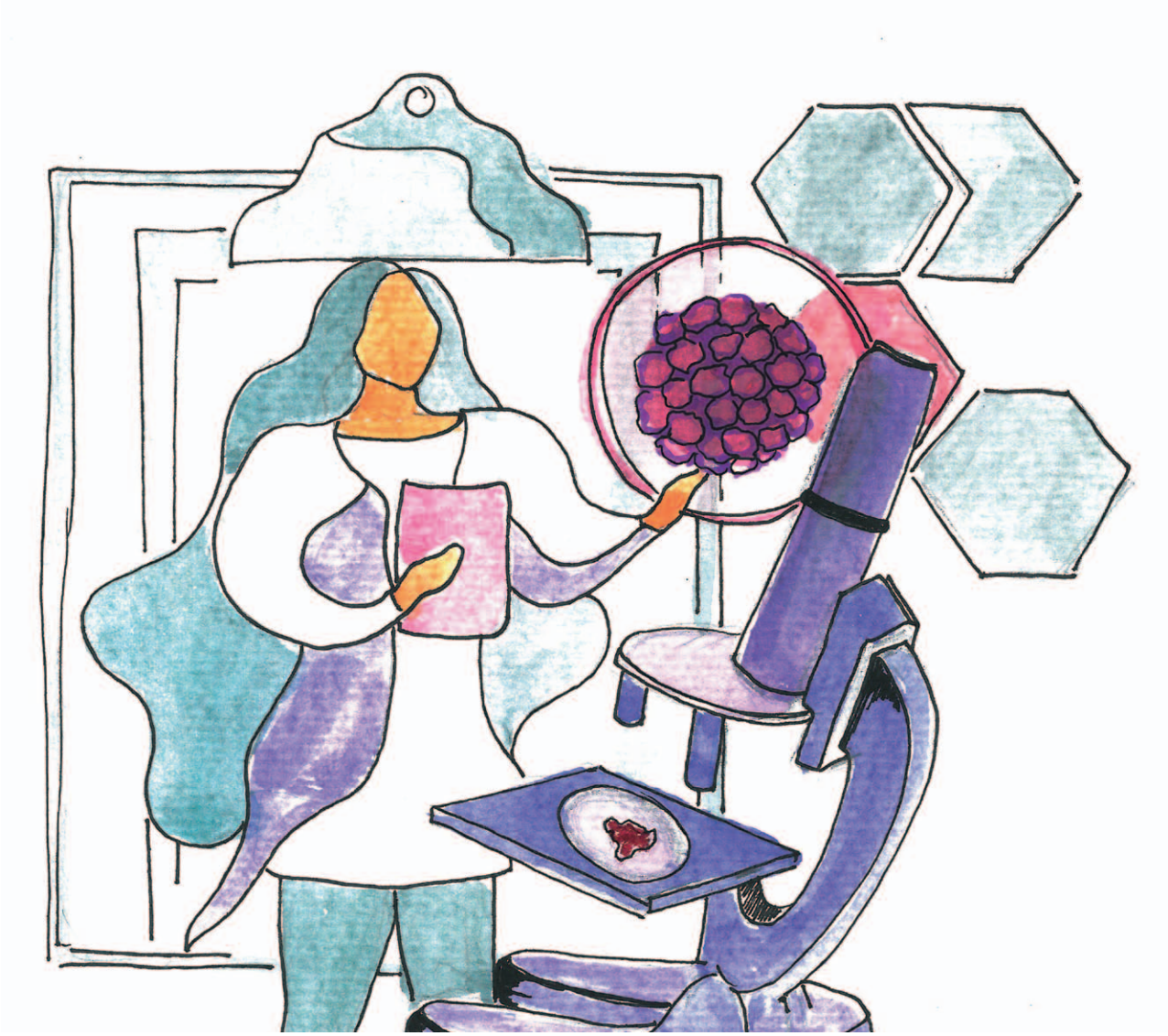
The use of the HPV test in a screening system also varies from country to country, but the world is moving in one direction. According to the WHO recommendation, a good quality HPV test should be used first and foremost from an appropriate age. In practice, this means that for people over the age of 30, HPV testing should be taken as primary screening.

If a test is **NEGATIVE**, most people can wait to re-test for 3–5 years. After all, there is no HPV infection that would otherwise develop into a lesion this quickly.

If the test is **POSITIVE**, the question is whether the infection is transient or transformative. This is determined by biomarker tests.

As we said above, Pap smears are most often used for this purpose (the triage of HPV positives for gynaecologic and colposcopic exam). Some biomarkers (methylation markers, dual stains with p16 or Ki-67), HPV genotypings, and the presence of some cancer-making proteins (E6–E7) are under investigation and may be used to fine-tune the triage compared to having smear alone (fewer women will be referred and so fewer women will be anxious about having a positive result).

Two proteins should be highlighted in this process: p53 and retinoblastoma (RB). P53 prevents uncontrolled cell division, while RB stops the cell cycle for control. HPV proteins are E6 and E7 viral proteins. E6 inhibits p53 in its function, thus preventing cell cycle arrest, possibly cell death. E7 inactivates the retinoblastoma protein. The virus results in uncontrolled, and error-free division of epithelial cells and the formation of abnormal tissue proliferation, while the virus seeks only to multiply on its own. **(5)**



Different types of HPV tests

There are several HPV tests. This field is evolving quickly, with more companies coming out with new products. This is beneficial for both men and women.

But are the tests good enough? Can we trust them?

In the context of HPV-based cervical cancer screening, it is crucial to only use tests that are sufficiently validated to ensure high-quality performance.

Of course, this is not to be decided by laypeople. The science, as seen by the several existing protocols and guidelines (FDA approval, Meijer Protocole, Valgent protocol), is always fine-tuning the answer to this question. The ESGO Prevention task force proposes eight other assays that can be considered as usable in cervical cancer screening (in alphabetic order), besides the two standard comparator assays (HC2 and and GP5+/6+ PCR): **(6)**

- Alinity m HR HPV Assay [Abbott, Wiesbaden, Germany]
- Anyplex II HPV HR Detection [Seegene Seoul, South Korea]
- Cobas 4800 HPV Test [Roche Molecular System, Pleasanton, CF, USA]
- HPV-Risk Assay [Self-Screen BV, Amsterdam, The Netherlands]
- Onclarity HPV Assay [BD Diagnostics, Sparks, MD, USA]
- PapilloCheck HPV-Screening Test [Greiner Bio-One, Frickenhausen, Germany]
- RealTime High Risk HPV Test [Abbott, Wiesbaden, Germany]
- Xpert HPV [Cepheid, Sunnyvale, CA, USA]

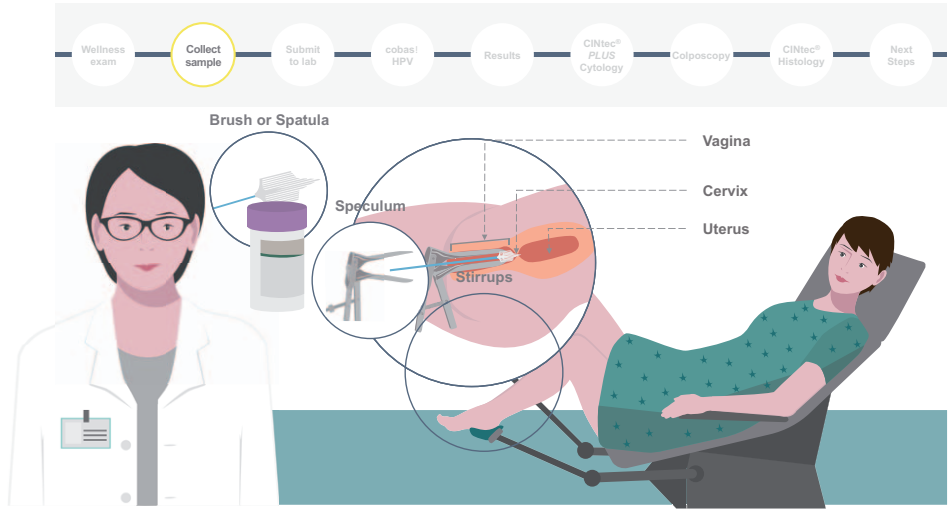
However, based on the ESGO review, there are no clinically significant differences between DNA and RNA tests for primary cancer screening with respect to both sensitivity and specificity.

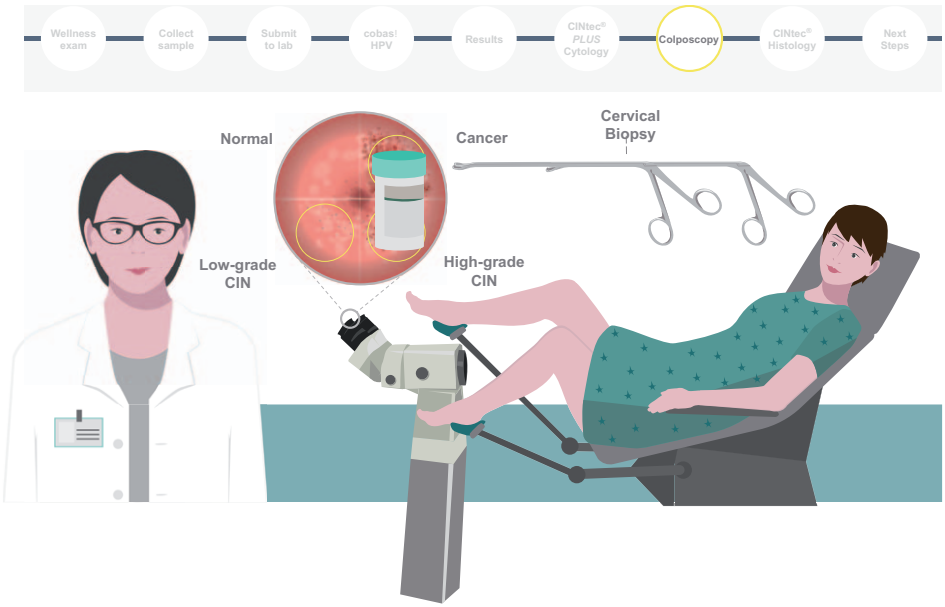
How can sampling be done?

1. Cytology – PAP smear test

The HPV test is a smear taken by a doctor. For smears, the doctor (in some countries, a general practitioner, a physician's assistant, a nurse, health visitors) takes a swab from the cervix and then fixes it for lab testing. At the laboratory, it is examined by a special machine and/or cytopathologist. Pap smears can be conventional (cells are sampled on a glass slide) or in a liquid (liquid-based cytology-LBC). LBC is suggested to clear some unwanted cells in the taken samples, such as blood or mucus, and may be a better sampling for evaluation by cyto-pathologists.

HPV can also be evaluated from the LBC samples by using some HPV tests (known as reflex HPV testing). But this is not possible for conventional smears.



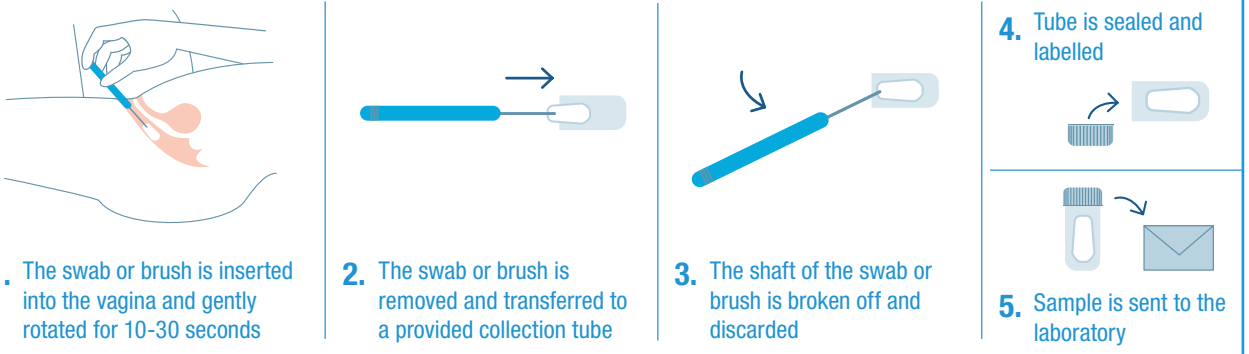


2. HPV test

3. HPV self-test

Self-collection of a sample for cervical cancer screening is done at home by the patient, who swabs the vagina, as shown in the picture below.

Self-collection of a sample for cervical cancer screening by swabbing the vagina[1]



1. The swab or brush is inserted into the vagina and gently rotated for 10-30 seconds
2. The swab or brush is removed and transferred to a provided collection tube
3. The shaft of the swab or brush is broken off and discarded
4. Tube is sealed and labelled
5. Sample is sent to the laboratory

Cytology results definitions (Cytology Dictionary)

- ASCUS: Atypical Squamous Cells of Undetermined Significance.

  The appearance of the cells is different from normal ones but not abnormal.
- ASC-H: Atypical Squamous Cells cannot exclude HSIL.
- LSIL: Low-grade Squamous Intraepithelial Lesion. Mild squamous cell lesion.
- HSIL: High-grade Squamous Intraepithelial Lesion. Serious squamous cell lesion.
- The CIN 1 lesion is not yet considered a pre-cancerous condition but increased monitoring is warranted.
- CIN 2 and CIN 3 are pre-cancerous conditions.

References

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(2-4) *1. Ronco G, Dillner J, Elfstrom KM, et al. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. Lancet 2014; 383(9916): 524-32. 2. Arbyn M, Ronco G, Anttila A, et al. Evidence regarding human papillomavirus testing in secondary prevention of cervical cancer. Vaccine 2012; 30 Suppl 5: F88-F99. 3. Sankaranarayanan R, Nene BM, Shastri SS, et al. HPV screening for cervical cancer in Rural India. NEnglJMed 2009; 360(14): 1385-94.*

(5) *Dr. Edina Lukács, Icó Tóth, Mallowpacket-HPV Book, 2020. Budapest, page 19-20.*

(6) *2020 ESGO list of hpv assays that can used for cervical cancer screening, M. Arbyn; M. Gultekin*

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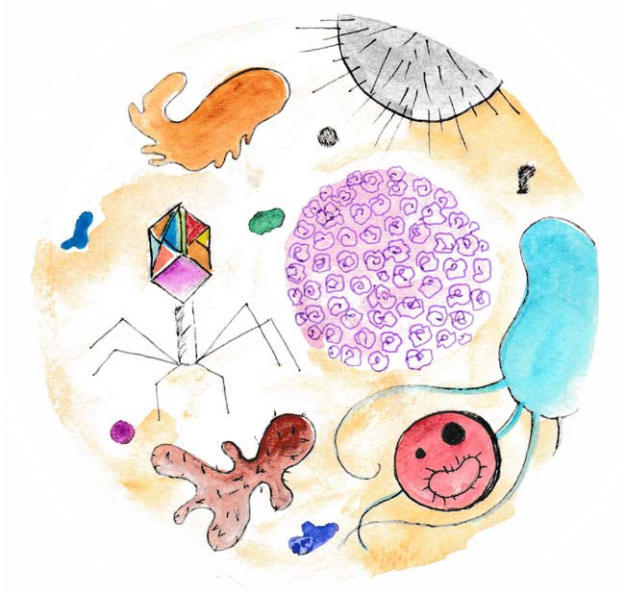
ENGAGe recommends contacting your local patient association!



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