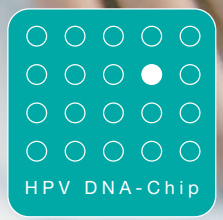
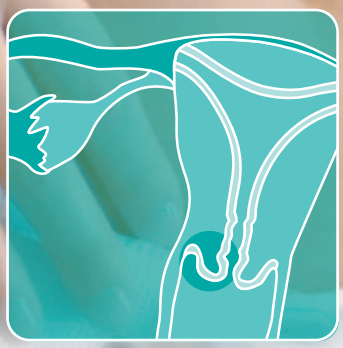




Don’t rely on intuition – Be certain

PapilloCheck® HPV-Screening

Type-specific identification of 24 types of the human papillomavirus

greiner bio-one

Cervical carcinoma

The risk is calculable

1. It has been scientifically proven that high-risk types of the human papillomavirus (HPV) cause more than 99% of all cervical carcinomas.

2. Since cervical carcinomas in the early stages are successfully treated in nearly 100% of all cases, an early detection of the causative virus is crucial.

3. High-risk types of the human papillomavirus differ in their propensity in triggering cervical cancer.

4. HPV genotyping thus permits an individual assessment of the cervical cancer risk of a patient.

PapilloCheck®

Individual cervical carcinoma screening

1. PapilloCheck® identifies 24 types of the human papillomavirus. A definitive determination of the HPV types present makes it possible to predict the risk of cervical carcinoma to the patient.

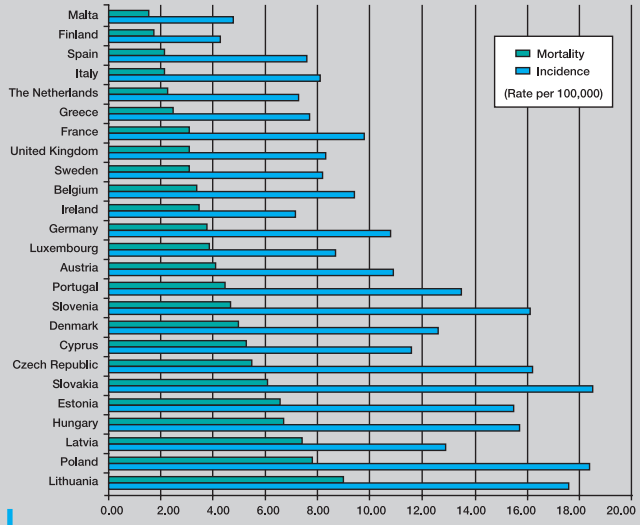
2. Regular type-specific screening for acute and persistent HPV infections with PapilloCheck® allows the progression of the infection to be accurately monitored.

3. In the context of an HPV vaccination PapilloCheck® provides clarity about the HPV status of the patient.

4. With a sensitivity of 98% PapilloCheck® delivers clinically relevant results. This highly predictive result is obtained quickly and reliably.

1. Incidence of cervical carcinomas

Cervical carcinoma is the second most common type of cancer in women, causing 233,000 deaths **worldwide** per year. Nearly 471,000 new cases are reported annually, however substantial regional differences in incidence and mortality are observed.[1]



Incidence and mortality of cervical carcinomas in Europe[2]

In **Europe** approximately 60,000 new cases and nearly 30,000 deaths are recorded annually. Thus cervical cancer takes second place after breast cancer as a cancer-related cause of death in Europe among women under 45 years of age.[3] Moreover, owing to the differences in screening or early detection programmes, the incidence rate is country-specific. Here a clear east-west gradient can be discerned. While in the Western European countries the number of deaths per 100,000 women fluctuates at a relatively low level between 1.8 (Finland) and 5.0 (Denmark), the average in the Eastern European countries is relatively high with about 7.2 deaths per 100,000 women.

The **average age at disease onset** for cervical carcinoma, i.e. age at diagnosis, is 52 years. Thus, in comparison with many other cancers, cervical cancer strikes many women at a relatively early stage in life.

Average age at disease onset by cancer type:

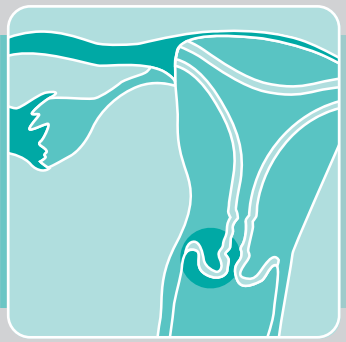
Ovarian cancer	*68 years*
Corpus uteri cancer	*67 years*
Breast cancer	*62 years*
Cervical cancer	*52 years*

For most types of cancer the **incidence of contracting the disease** grows with increasing age. This is not true for cervical carcinoma. Here, substantially more women are diagnosed with cervical cancer between the ages of 25 and 35 years than those over 65. This represents an age incidence with a first peak between 35 and 55 years, which is then followed by a second increase in the incidence rate after about 60 years of age.[4]

Curability

Cervical cancer strikes women in a relatively early phase of life.
However, independent of the age of the patient, in precancerous early stages cervical carcinoma can be successfully treated in nearly 100% of all cases!

2. Status quo of early detection screening



Since early stage cervical carcinomas are nearly 100% curable, early detection by screening programmes for gynaecological cancers is of great importance.

For this reason in most countries exist national screening and prevention programmes, albeit with very different efficiencies. Finland, for instance, has the lowest incidence rate for cervical cancer with a screening interval of 5 years.

Cytological examination using the Pap smear

In the framework of the European early detection programme the cytological investigation of the *cervix uteri* developed in the mid 1940's by Papanicolaou and Traut is regarded as the "Golden Standard". Since the so called Pap test, named after the "inventor", has been incorporated into routine cancer screening programmes, the annual rates for detection of new cases and mortality in Europe have decreased markedly.

However, the Pap cytology test is limited in its predictive value. This is because although it recognises symptoms, it is unable to detect the causative agent, namely a persistent infection with a cancer causing human papillomavirus (HPV).

Moreover, in recent years, numerous published international and national studies show **deficiencies with respect to the quality and efficiency** of early detection of cervical cancer using the Pap smear.[5–9]

Golden Standard

The "Golden Standard" of early detection screening is the Pap cytology but it turns out to have shortcomings in its sensitivity and it only detects the symptoms. Modern procedures are focussed on the trigger of cervical carcinomas, the human papillomavirus.

Quality deficits of the Pap smear:

1. **Inadequate sensitivity**
   In a recent report from the USA, an average **sensitivity of only 50%** was determined. Reasons for the inadequate sensitivity of the cytology include a lack of quality assurance measures, improper collection of samples and erroneous findings.[5]

   This low sensitivity is also one of the reasons for the observed stagnation in the fight against cervical cancer.

   Thus currently every second woman who has regularly gone to cancer screening will be afflicted with the disease because of a false negative cytology!

2. **Unsuitable for adenocarcinomas**
   In contrast to an HPV test, the Pap smear is not suited for the detection of the less common adenocarcinomas of the *cervix uteri*.

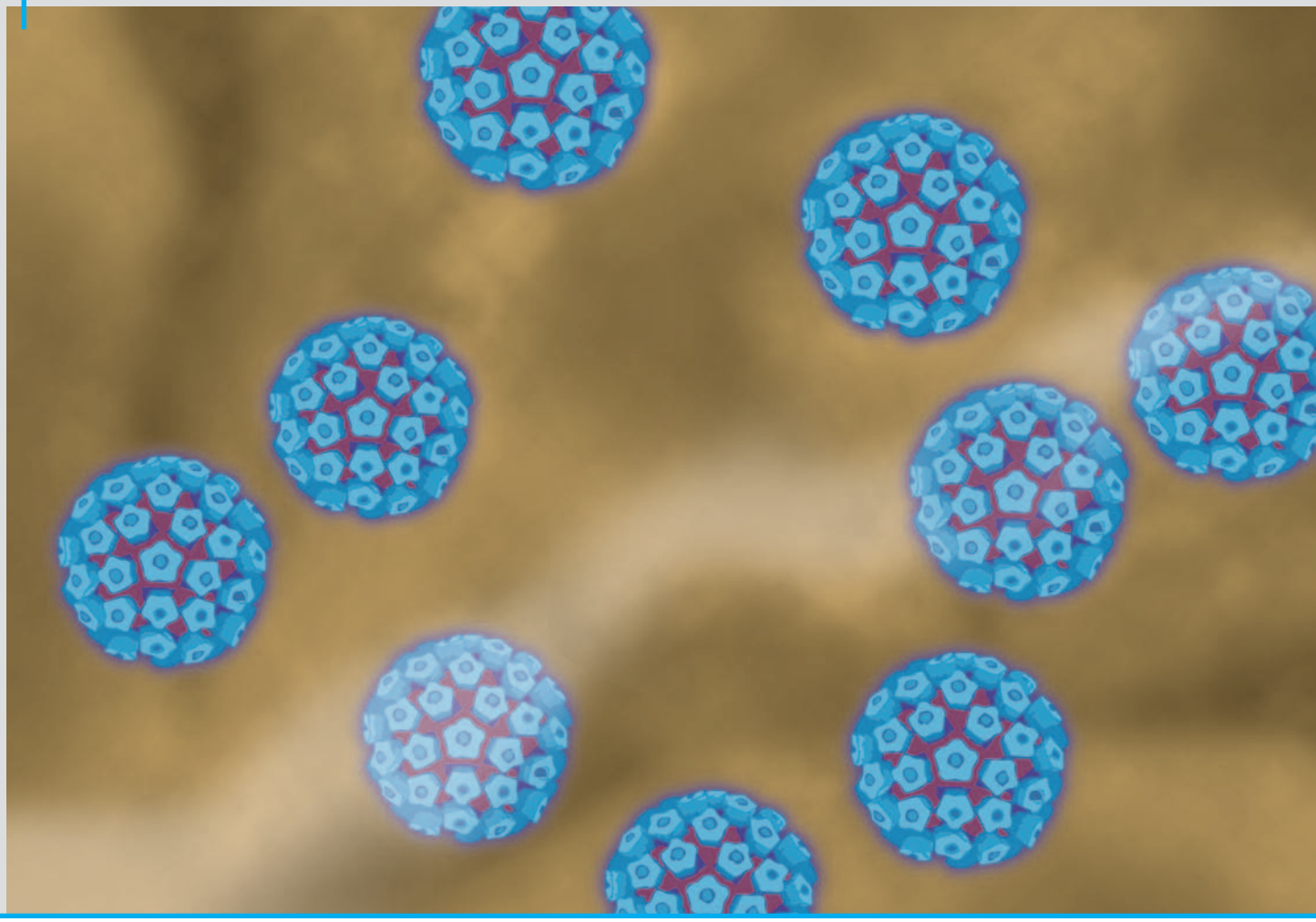
3. Formation of cervical carcinomas

The trigger: Human papillomaviruses

Today it is scientifically established that certain types of the human papillomavirus (HPV) can infect the cells in the genital tract and thereby represent the major cause of cervical cancer. Well over 100 HPV types are known thus far.[10–13]

Prevalence of human papillomaviruses
Human papillomaviruses are widely prevalent. An estimated 75 to 80% of all women and men become infected with genital HPV sometime in the course of their lives. Every infected person can transmit the virus to other people through genital contact, primarily through sexual intercourse. Thus a short-lived HPV infection may be regarded as a quite normal consequence of sexual activity.
The frequency peak for detectable HPV infections lies in the age group between 20 and 25 years. With increasing age the prevalence of detectable HPV infections decreases.[14]

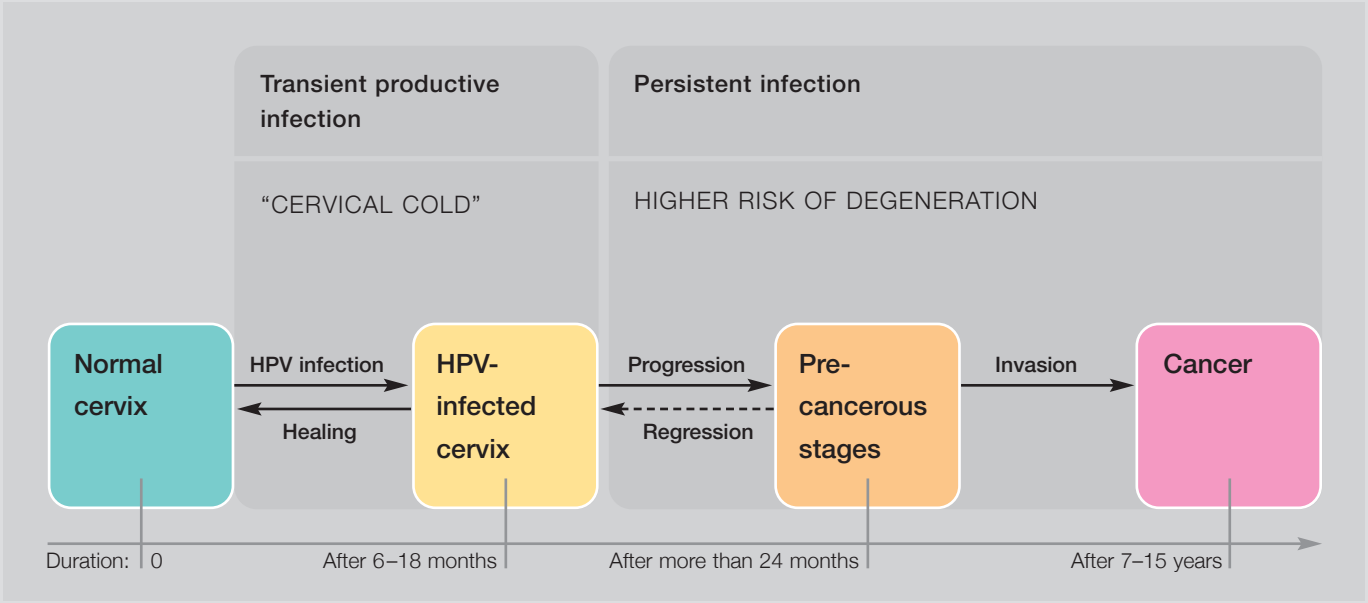
Human papillomaviruses



Characteristics of an HPV infection

An HPV infection is in most cases eliminated by the body's immune system over a time period of about 8 to 14 months. This temporary or transient infection, in analogy to a viral infection, e.g. of the nasal passages, is also termed as "cervical cold". As a rule it runs its course and regresses without any symptoms. Pain or other signs do not appear, so that the HPV infection is not noticed by most women.[15–19]

Only in a few cases does the infection remain (persistent infection) and it can then lead to precancerous early stages (dysplasias, CIN=Cervical Intraepithelial Neoplasias). However, even then, it is not a foregone conclusion that a cervical carcinoma will arise. In many cases the early stages regress, i.e. are healed. The self-healing rate for mild or moderate dysplasias (CIN1 or CIN2) fluctuates between 40 and 90%. Thereby, along with a number of so called cofactors, the age of the woman at the time of diagnosis is quite crucial. The younger the woman at diagnosis, the better the prognosis for a spontaneous regression of the cancer precursor stages. If the precancerous stages themselves remain undetected, a cervical carcinoma can develop over a relatively long time period of at least 7 years and up to a decade after the original HPV infection.[20]



Cofactors

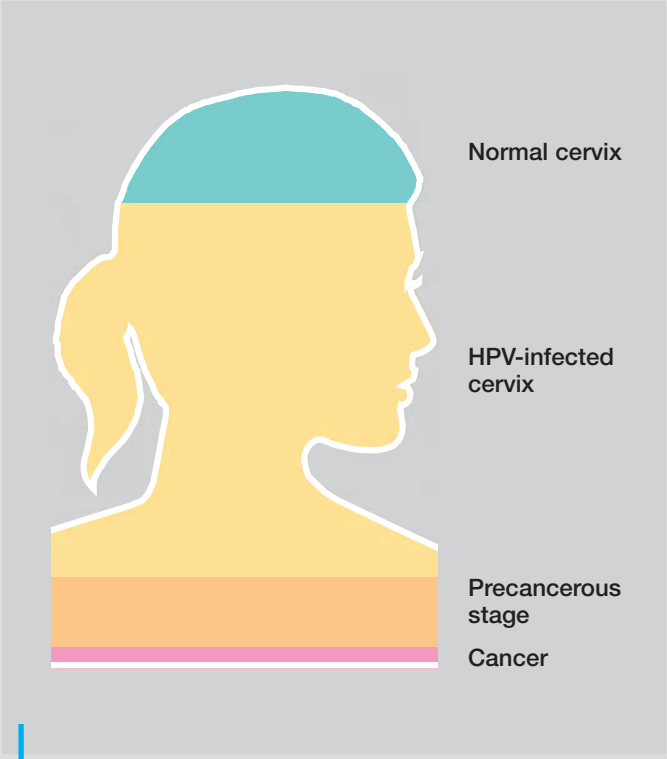
Infection with high-risk types is a necessary precondition for the appearance of a cervical carcinoma. Besides age, however, the following cofactors can further increase the risk of disease:

- Smoking
- Further infections in the genital tract
- Numerous pregnancies and premature births
- AIDS
- Medications which suppress the immune system, e.g. after an organ transplantation
- Taking a hormone contraceptive ("the pill") for a longer period of time

Persistence

A persistent, long lasting infection with high-risk types of the human papillomavirus is a necessary prerequisite for the development of a cervical carcinoma.

An HPV infection does not necessarily mean cancer!



Probability of the development of cervical carcinomas or precancerous stages

An estimated 75 to 80% of all women become infected with genital HPV at some time over the course of their lives. For many HPV infected women (80%), the body's natural immune defence can eliminate the infection after a period of approximately 12 months.
For 20%, however, persistence or progression is observed. For 5 to 10% of all high-risk HPV positive women – with most between 20 and 40 years of age – cytological abnormalities develop.
Then, after an interval of 7 to 15 years less than 1% of the persistent high-risk HPV infections lead to a carcinoma.
Once an infection has occurred, the establishment of regular HPV screening ensures an accurate monitoring of the infection and the ability to predict the course of the disease.

Disease progression

The number of women for whom a cancer precursor stage is detected is about 100-fold as high as the number who actually develop a cervical carcinoma. Regular HPV checkups give certainty about the course of the disease.

The HPV type is crucial!

The genital **human papillomavirus** types are divided into two groups. The classification is made according to their propensity to trigger cancer growth:[21]

Low-risk types, (low-risk HPV or lr-HPV)
Low-risk types cause mainly genital warts (genital condylomas) and are practically never found in cancer patients. HPV 6 and – less frequently – HPV 11 are the main causative agents (approx. 90% of all cases) of warts in the genital area.

The most common low-risk HPV types

6 11 42 43 44

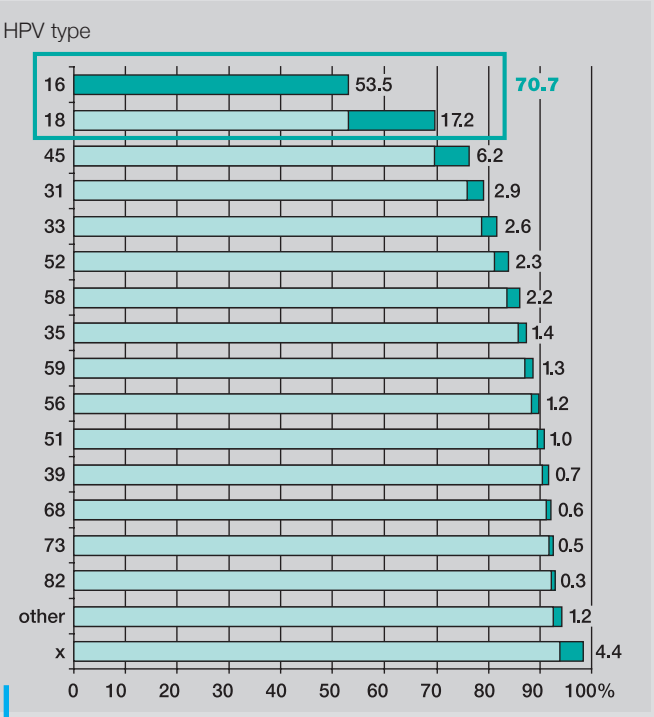
High-risk types (high-risk HPV or hr-HPV)
High-risk types cause precancerous stages (dysplasias, cervical intraepithelial neoplasias, CIN) and cancer. They are **identified in 99.7% of all cases of cervical carcinoma**. A majority of cervical carcinoma cases (approx. 70%) are triggered by just two hr-HPV types: **HPV type 16** (53.5%) and **HPV type 18** (17.2%).[22]

The most important high-risk HPV types

16 18 31 33 35 39 45

51 52 53 56 58 59 66

68 73 82



Contribution in percent of the different high-risk HPV types to the occurrence of cervical carcinomas[22]

Risk potential

Scientific investigations show that:
Although for a persistent infection with a high-risk HPV type the risk of contracting a cervical carcinoma is generally increased, certain high-risk HPV types show a particularly high risk potential.

The risk is calculable!

Depending on the high-risk HPV type, the probability that cancer growth is triggered is increased by up to 400-fold (HPV type 16). A persistent infection with HPV type 68 in contrast increases the probability by "only" 50-fold.[23]
High-risk HPV types show differing degrees of virulency. However, over 70% of all cervical carcinomas can be attributed to persistent infections with HPV types 16 and 18. On this background type-specific, persistent infection with types 16 and 18 takes on particularly great importance.[24–26]

A 10 year study in Denmark showed that women with an initial normal Pap smear, but a persistent infection with HPV 16, have an absolute risk of 52% of contracting a cervical carcinoma with severe dysplasia within 10 years. By contrast women with a persistent infection with another HPV type other than HPV type 16 or 18 had a risk of only 13%.[27]

With the aid of HPV genotyping, the prediction of the likelihood of developing cervical cancer can be managed more effectively. So, type-specific HPV testing should be considered a fundamental and necessary prerequisite for the risk evaluation of a positive sample.

"The clinical management of patients in an early detection screening system, comprising cytology and an HPV test, demands the addition of HPV type-specific detection procedures. Through the combination with a temporally downstream test for type-specific virus persistence, a much needed reform of the early detection screening system is possible with longer examination intervals for women aged over 30 years without leading to any unacceptable increase in colposcopy examinations and an overtreatment of the women."

Prof. Dr. rer. nat. Thomas Iftner, Institute for Medical Virology, University of Tübingen

New chances against cancer: HPV genotyping and HPV vaccination!

The development of preventive vaccines against the two most "aggressive" high-risk HPV types 16 and 18 represents a major advance in the fight against the occurrence of cervical cancer. But this also requires future participation in cancer early detection screening examinations:[28–32]

- The vaccine protects solely against diseases (warts and cancer) triggered by human papillomavirus of low-risk types 6 and 11, and high-risk types 16 and 18. But 30% of all cervical carcinomas are triggered by other high-risk HPV types.
- Up until now it remains unclear whether vaccinated women will be infected more frequently with those HPV types which are not involved in the vaccine (so called type replacement).

In this context HPV genotyping becomes particularly important. It provides clarity about the HPV status of a patient both before and after an HPV vaccination.

HPV genotyping

HPV genotyping permits a better evaluation of the risk potential of an HPV infection for triggering cancer. In addition a persistent HPV infection can thereby be detected and identified type-specifically.

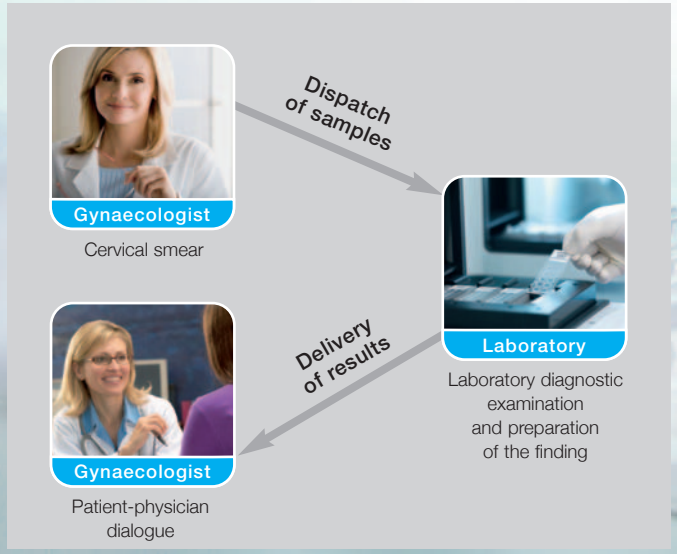
4. HPV genotyping with PapilloCheck®

In order to fulfil the need for an improved risk evaluation for HPV infections, Greiner Bio-One has developed the DNA array PapilloCheck®. PapilloCheck® is certified in the European Union (CE) as an in vitro diagnostic (IVD) for the qualitative type-specific identification of 24 human papillomavirus types from a cervical smear. With the test a total of 6 low- and 18 high-risk HPV types can be detected. With a sensitivity of 98% PapilloCheck® provides a maximum level of reliability and substantially better chances of a cure.

HPV types identifiable with PapilloCheck®:

Identifiable low-risk HPV types	Identifiable high-risk HPV types
6	16
11	18
40	31
42	33
43	35
44	39
	45
	51
	52
	53
	56
	58
	59
	66
	68
	70
	73
	82

From smear to finding



After the collection and sending off of the cervical smear by the gynaecologist the human papillomavirus is detected in the laboratory using the DNA array. The digital evaluation of the DNA array facilitates the rapid subsequent preparation of a report as well as sending the finding to the gynaecologist electronically. The type-specific PapilloCheck® result makes it possible for the gynaecologist to consider the individual risk potential of the patient.

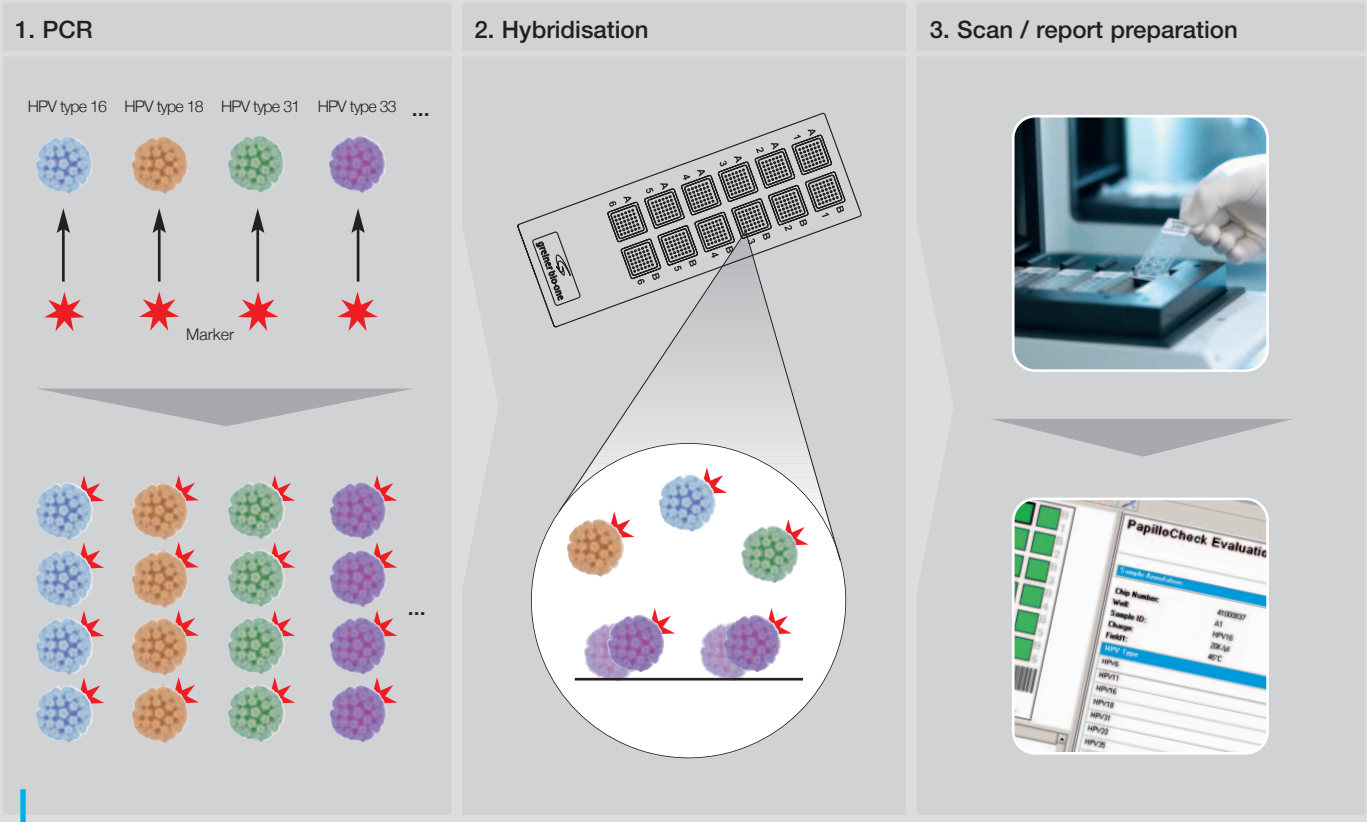
PapilloCheck® detection

Through its type-specific HPV identification PapilloCheck® provides the gynaecologist with an individual estimation of the cervical carcinoma risk of the patient. With a sensitivity of 98% PapilloCheck® further provides a maximal degree of reliability.

Laboratory diagnostic analysis

PapilloCheck® is a probe test, which is utilised in conjunction with an extremely sensitive and rapid hybridisation technique, the so called DNA array. This enables a substantially faster and more exact identification of existing HPV infections in cervical smears.

In contrast to previous detection methods the analysis uses molecular biology techniques with a DNA array that is analysed in a computer controlled, high-resolution optical microarray scanner. This ensures reproducible and objective results of samples.



Schematic representation of the PapilloCheck® test principle

After collection of the cervical sample the viral DNA is extracted in the investigating laboratory. (1) In the following a specific DNA fragment from the E1 gene of all 24 HPV types is amplified in the presence of HPV-specific primer pairs with the help of the polymerase chain reaction (PCR). Thereby single stranded DNA fragments arise, which are then labelled with fluorophore molecules. (2) Then follows the hybridisation (binding) of the labelled amplified pieces of DNA onto HPV type-specific DNA probes from this region of the E1 gene, which are fixed on the PapilloCheck® DNA array. (3) After the binding the analysis by the computer controlled CheckScanner™ is performed fully automatically. A total of 5 control systems prevent false positive or false negative results. The evaluation and preparation of the PapilloCheck® Report are done with the aid of special analysis software. Thanks to the digital evaluation all results can be simply archived and electronically managed and delivered.

PapilloCheck® Report

After the laboratory diagnostic examination of the cervical smear and digital evaluation, a rapid and reliable representation of the highly predictive finding is prepared.

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