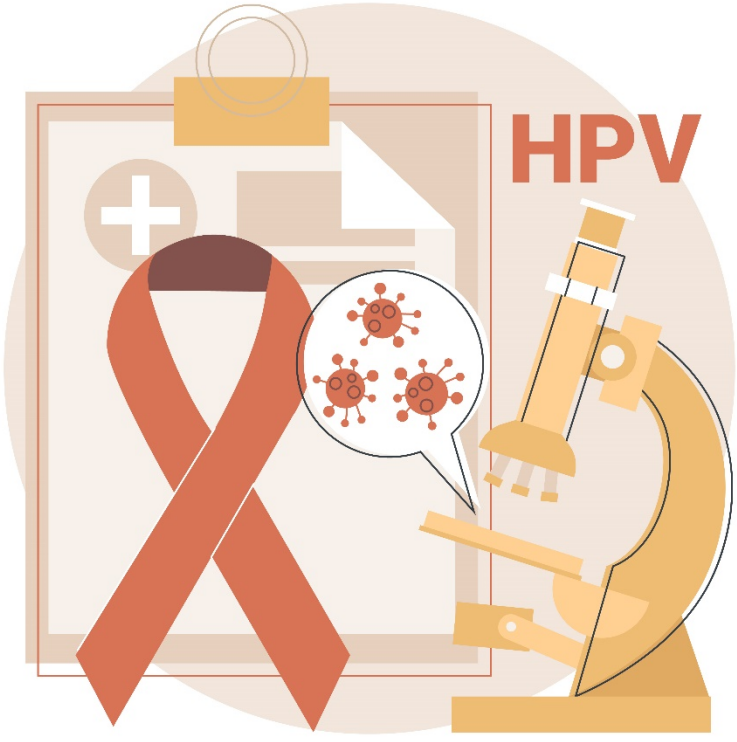
DNK s.r.o.
diagnostické centrum

HPV testing
for cervical cancer screening

by Real-Time PCR



Cervical cancer

Cervical cancer is one of the most common malignant tumors in women: annually more than 500 thousand new cases are detected in the world, more than half of them are fatal.

Human papillomavirus is the cause of cervical cancer in 95-99% of HPV cases

More than 80% of women become infected with HPV during the first years after the start of sexual activity

In 80 % of those infected, the virus is excreted from the body within 2 years

In about 10% of cases, the infection becomes chronic with risk of precancerous lesions and invasive cervical cancer.

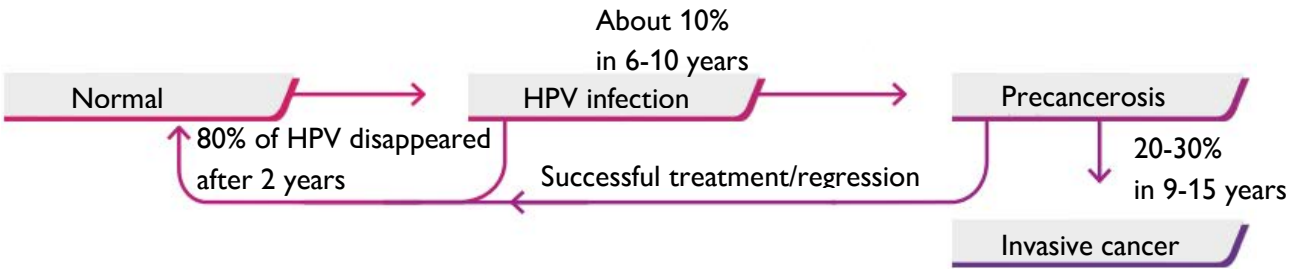
In accordance with WHO, HPV types are classified according to the degree of their oncogenicity.

High carcinogenic risk types: 16 and 18 - the most oncogenic; 31, 33, 35, 39, 45, 51.52, 56, 58, 59, 66, 68

Low carcinogenic risk types: 6, 11, 42, 43, 44

Types of likely carcinogenic risk: 26, 53, 67, 70, 73, 82

Other: About 200 types



In most cases, cervical cancer develops for a long time, without the manifestation of clinical symptoms. The disease is detected in the late stages, when the percentage of recovery of patients is extremely low.

Cervical cancer can be successfully prevented if changes in the precancerous stage are detected.

WHO has developed a set of preventive measures aimed at reducing the incidence and mortality of cervical cancer:

Primary prevention	Secondary prevention	Tertiary prevention
• HPV vaccination for children aged 9 to 14 years • Health education, sexuality education for children and adolescents	Cervical cancer screening from 21 to 69 years: • HPV testing • Cytology Timely treatment of precancerous lesions	Treatment of cervical cancer for all women, if necessary: • Surgical treatment • Radiotherapy • Chemotherapy

Laboratory diagnostic methods in cervical cancer screening:

Cytological method: examination of swabs taken from the cervix and cervical canal

- The main method of cervical screening due to availability and fairly high specificity (63-99%) for detecting precancerous changes
- It has low sensitivity (50-70%) due to incorrect sampling of specimen and subjective evaluation of results, as well as the presence of samples with cytological changes that are difficult to classify
- Liquid cytology is a variant of the cytological method, which allows for better examination of the drug

PCR method: detection of HPV DNA types of high carcinogenic risk in swabs taken from the cervix and cervical canal. **Conducting this testing can significantly increase the effectiveness of cervical screening!**

- ✓ Increase in test sensitivity to 96.7%: Significantly more detected precancerous lesions compared to cytology itself
- ✓ Extension of intervals between repeated tests up to 5 years
- ✓ Reducing the economic costs of screening

Collection of specimen for the detection of HPV DNA and for cytological examination can be carried out simultaneously. Liquid cytological media can be used for PCR analysis.*

The following cervical screening approach is recommended:*

The screening is suitable for all women aged 21 to 69 years. Up to 29 years of age, only cytological examination once every 3 years is allowed due to the high risk of HPV infection, but the low probability of developing pathology. From 30 to 69 years of a woman's life, it is necessary to conduct a cytological examination and HPV testing at least once every 5 years. These tests may be carried out in parallel.

Features of the use of PCR in the diagnosis of HPV infection

• High sensitivity of the method and significant negative predictive value of a test result. At the same time, low specificity, since the presence of HPV not always leads to cervical cancer in the future.

- Possibility of application at any stage of cervical cancer screening
- If needed, PCR analysis allows genotyping and viral load determination
- Testing for the presence of HPV DNA is necessary in case of ambiguous results of cytology

RealBest Technology for HPV testing in combined cervical cancer screening

Using a single biological sample for HPV testing and liquid-based cytology (LBC)

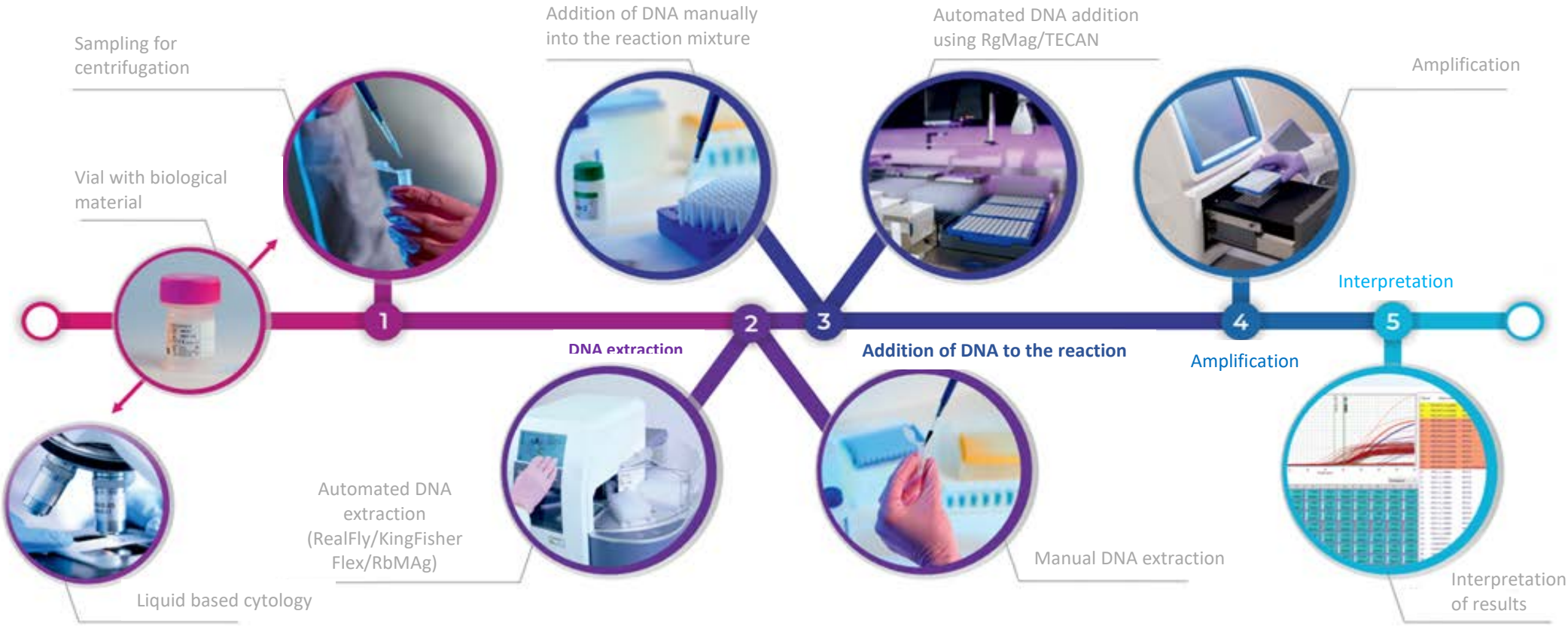
Detection of high-risk HPV, genotyping and determination of viral load

High-throughput capacity due to plate format and ready-to-use reaction mixture (Ready Master Mix)

User-friendly software

Automation of one or more steps of analysis

Service and methodological support



To add samples and reagents to high-profile tubes and also isolated DNA to the prepared reaction mixture, the following can be used:

- automated pipetting station RbMag/RealBest/TECAN Freedom Evo
- multichannel pipette

Recommendations for the collection of biomaterial

1. Do not take a swab during treatment for genital infections.

2. Testing should be carried out at least 48 hours after:

• sexual intercourse, the end of menstruation;

• prolonged colposcopy with treatment with a 3-5% solution of acetic acid or Lugol;

• using lubricants, tampons or spermicides.

3. A swab must be taken prior to bimanual examination.

4. Before collecting the specimen, clean the external opening of the cervical canal from mucus plugs and vaginal secretions with a sterile swab.

Collection of swab for PCR analysis

The collection is made using a urogenital probe or cytobrush. Rinse the working part of the cytobrush/probe containing the test material in a tube of transport solution, press against the walls of the tube and discard. Close the tube tightly with a cap.

Temperature and storage time:
• (18–26) °C not more than 2 days;
• (2–8) °C not more than 2 weeks;
• minus (18–40) °C not more than 2 months,
• below minus 40 °C – long-term.
Repeated freezing and thawing of samples is not allowed.

Collection of biological specimen for Liquid based cytology and PCR

It is made using a cervical brush with ectocervical and endocervical component. Insert the removable brush head together with the collected material into the vial with stabilizing solution, close the vial tightly.

The temperature and storage time are indicated in the instructions for cytological medium.

Freezing of samples is not allowed.

Validation of specimen collection

When testing epithelial cell swabs, there is a possibility of obtaining a false negative result in case of insufficient amount of specimen.
To increase the reliability of PCR analysis, it is recommended to use the "**RealBest Sample Validation**" kit:

Used to determine sufficient human DNA content in epithelial swabs.

It allows, if necessary, to determine the relative amount of pathogen in the sample.

RealBest® Technology: solution for HPV testing in cervical cancer screening

Collection of specimen

8894/8885 Transport solution (for PCR)

8899 RealBest DNA express

DNA extraction

Manual:

8899 RealBest DNA express

8896 RealBest extraction 100*

Automated:

8883 RealBest UniMag*

HPV DNA genotyping

8497 RealBest DNA HPV HR genotype

Differential detection of 12 high-risk HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 a 59

HPV DNA genotyping with quantification

8478 RealBest DNA HPV HR genotype quantitative

Differential detection and quantification of 12 high-risk HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 a 59

HPV genotyping

8475 RealBest DNA HPV 6/11
8473 RealBest DNA HPV 16/18
8459 RealBest DNA HPV 26/51
8471 RealBest DNA HPV 31/33
8469 RealBest DNA HPV 35/45
8447 RealBest DNA HPV 44
8448 RealBest DNA HPV 66
8488 RealBest DNA HPV 68
8449 RealBest DNA HPV 26/53/66
8451 RealBest DNA HPV 68/73/82
8446 RealBest DNA HPV 6/11/44

Screening

8444 RealBest DNA HPV Screen
Detection of 14 high-risk HPV genotypes (without genotyping): 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 a 68

8498 RealBest HPV Oncoscreen
Detection of 14 high-risk HPV DNA genotypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 a 68 with differentiation of genotypes 16 and 18 and determination of the total human DNA content of the sample

Validation

8888 RealBest Sample validation
Quantitative evaluation of human DNA content

* the kit is suitable for extraction of DNA from specimen taken into the medium for Liquid based cytology

The format of the kits is designed to perform 96 tests, compatible with plate amplifiers

Diagnostické centrum DNK, s.r.o.
Brestová 14, 821 02 Bratislava
+421 911 299 324, +421 911 211 404
dnk@pharma.sk, diagnostika@pharma.sk
www.pcr.sk