DNK s.r.o.
diagnostické centrum

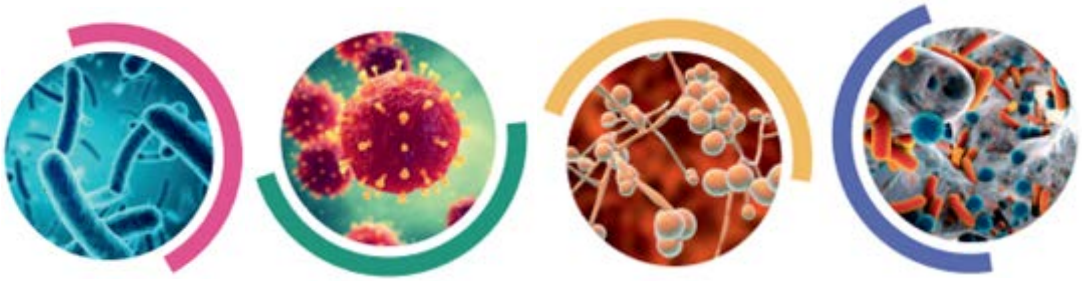
Laboratory diagnosis of sexually transmitted diseases

by Real-Time PCR

Sexually transmitted diseases

More than 30 bacterial, viral and parasitic pathogens are potential causative agents of sexually transmitted diseases (STDs). Their symptoms are nonspecific, moreover, all STIs can occur in an asymptomatic form and are not recognized by infected persons.
Such individuals can transmit infections to others and may be at risk of complications, which include reproductive disorders, infertility, complications during pregnancy or the neonatal period, and others. The earlier the infection is detected and treated, the greater is the chance of recovery without complications.

Bacteria **Fungi** **Mycoplasmas** **Protozoa** **Vírusy**



Obligate pathogens	**Viral infections**	**Fungi**	**Conditional pathogens**
Chlamydia trachomatis	High-risk HPV	*Candida albicans*	*Gardnerella vaginalis*
Trichomonas vaginalis	HSV 1, 2	*Candida glabrata*	*Mycoplasma hominis*
Neisseria gonorrhoeae	CMV	*Candida tropicalis*	*Ureaplasma species*
Mycoplasma genitalium	HBV	*Candida parapsilosis*	*Atopobium vaginae*
Treponema pallidum	HCV	*Candida krusei*	*Prevotella species*
Haemophilus ducreyi	HIV	*Candida guilliermondii*	*Streptococcus species*
		Candida famata	*Enterococcus species*

- **Pelvic inflammatory disease**
- **Oncological diseases**
- **Sterility**
- **Ectopic pregnancy**
- **Abortion**

- **Obstruction of the vas deferens**
- **Azoospermia**
- **Sterility**

- **Congenital malformations**
- **Intrauterine fetal death**
- **Inflammatory processes in various organs and tissues**
- **Psychomotor developmental delay**

***Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma genitalium* a *Trichomonas vaginalis* are obligate pathogenic microorganisms requiring treatment, regardless of the amount of pathogen and the presence or absence of clinical manifestations**

The main sexually transmitted pathogens

Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, Mycoplasma genitalium

More than 1 million sexually transmitted infections are caused worldwide every day.

STI

Most STIs are asymptomatic or have only mild symptoms that may not be recognized as sexually transmitted infections

It can have serious reproductive health consequences beyond the immediate impact of the infection itself (e.g. infertility, mother-to-child transmission)

- **large distribution in the population**
- **possible infection with several pathogens at the same time**
- **similar symptoms in diseases caused by different pathogens**
- **can lead to serious complications**

Laboratory diagnosis of sexually transmitted diseases (STD)

Molecular biology methods

Microbiological methods

Bacteriological methods

Immunological methods

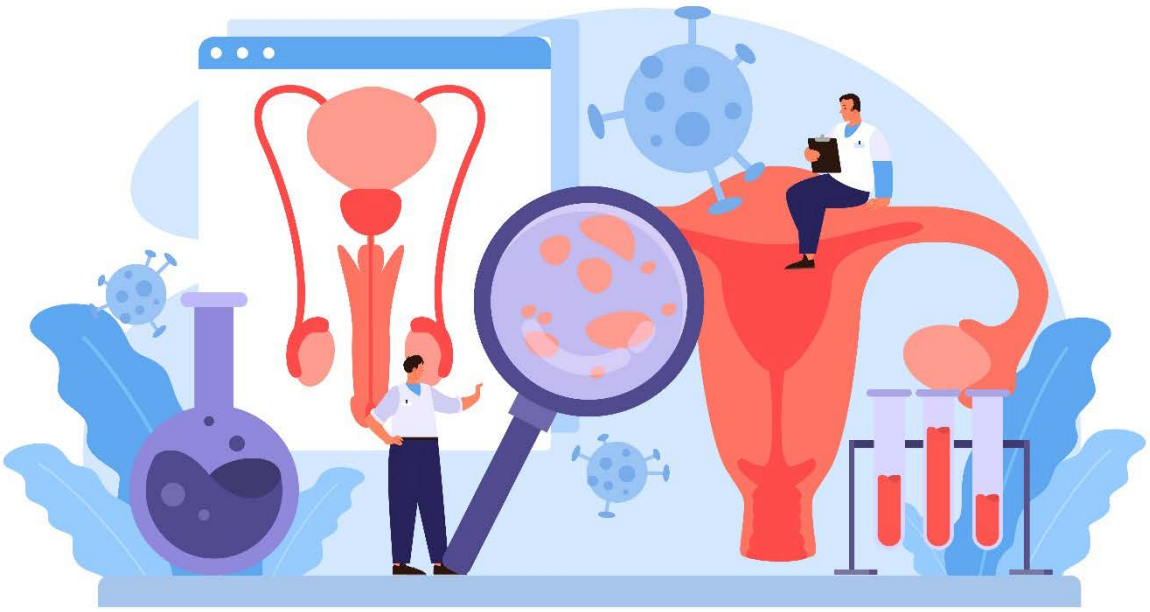
Due to the improved accuracy of the test, sample processing and ease of screening, the PCR method has great potential in the diagnosis of STDs:

PCR method options

- Determination of the etiology of the disease, including identification of mixed infections
- Screening for asymptomatic forms
- Genotyping, identification of bacterial strains
- Monitoring the efectiveness of treatment

Indications for testing for sexually transmitted infections

- Clinical and/or laboratory signs of inflammation of genitourinary organs
- Examination of sexual partners at the stage of pregnancy planning
- Screening women during pregnancy
- Upcoming surgical (invasive) manipulations on the pelvic organs
- Perinatal loss and impaired obstetric and gynecological history, infertility
- Sexual contact with a sick STI or anonymous/unverified partner



Chlamydia trachomatis – gram-negative bacterium, obligate intracellular parasite

- It affects the genitourinary system, but can infect lymph nodes, lungs, conjunctiva, synovial membranes of joints, ligaments, fascia
- It causes urethritis, cervicitis, salpingitis, trachoma, lymphogranuloma venereum, neonatal pneumonia and conjunctivitis
- In most cases, acute infection is replaced by chronic, and in 80% of cases it is asymptomatic in men and women

Chronic asymptomatic infection
Intracellular parasitism without conflict with immune system

Loss of motility of trichomonads outside the human body
The presence of various atypical (non-flagellate) forms

Important to consider when choosing a method of laboratory diagnosis

Trichomonas vaginalis – anaerobic, motile, flagellate element

- It affects the genitourinary system, but can infect the respiratory tract
- It causes urethritis, cervicitis and vaginitis in women, can cause complicated pregnancy, premature birth and low fetal weight
- Asymptomatic transmission is more common in men

Mycoplasma genitalium – the smallest pathogenic bacterium, no cell wall, intracellular parasite

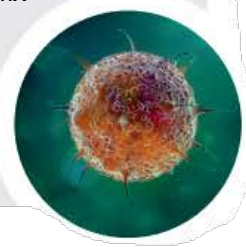
- It affects the epithelial cells of the urogenital and respiratory tract
- It causes urethritis, vaginitis, salpingitis and cervicitis. Possible development of inflammatory diseases of the pelvic organs, epididymitis, prostatitis, cystitis, pyelonephritis, secondary infertility
- Poorly recognized by the immune system, it provokes autoimmune diseases
- 40% of cases are asymptomatic in men and women

Smallest microorganism size and absence of cell wall
High antigenic variability due to the similarity of the structure of the cell membrane of mycoplasma with the cell membranes of macroorganisms
difficult to cultivate due to the small size of the genome
Antigenic similarity between *M.genitalium* and *M. pneumoniae*

Possibility of horizontal transfer of any genes between *N. gonorrhoeae* and comensal *Neisseria*, as well as between *Neisseria* and other bacteria of related species
Possibility of loss of almost any gene by individual strains of *Neisseria*

Neisseria gonorrhoeae – gram-negative immobile bacterium

- Colonizes the mucous membranes of the genitourinary system, anorectal region, conjunctiva, musculoskeletal system, oropharynx
- It causes urethritis, cervicitis, proctitis, salpingitis. It can lead to brain abscess, arthritis, endocarditis, meningitis, myocarditis, pericarditis, peritonitis, pharyngitis, pneumonia, sepsis and skin lesions
- For women, asymptomatic transmission is typical



The pre-analytical phase is very important for proper PCR testing

- Biological material must be taken from sites of alleged localization of the pathogen
- Transportation of biomaterial to the laboratory must be carried out under certain temperature conditions and storage period.
- To check the effectiveness of therapy, it is necessary to adhere to the deadlines for repeated sampling of biomaterial.

Material for laboratory testing

In women:

- discharge (smear) from the urethra, cervix, vagina, lower part of the rectum
- first-morning urine
- **for indications –** discharge from the oropharynx, large vestibular and paraurethral glands, mucous membrane of the eye

In men:

- discharge (smear) from the urethra
- prvá porcia ranného moču
- **for indications –** discharge from the lower rectum, oropharynx, mucous membrane of the eye, prostate secretion

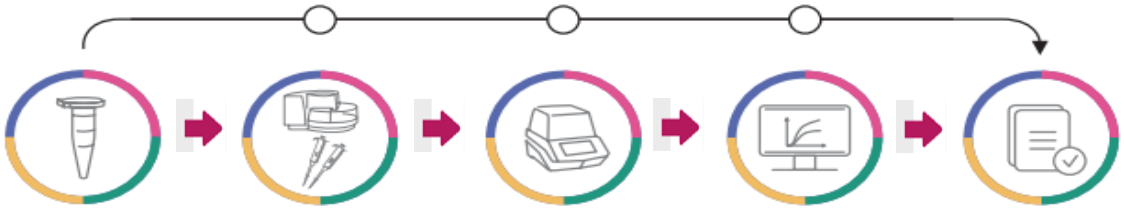
In newborns:

- bronchoalveolar lavage
- mucus
- **for indications –** discharge from oropharynx, conjunctiva

To check the quality of epithelial cell swab collection, it is necessary to use the "RealBest Sample Validation" kit

- The principle of validation of the quality of specimen collection is based on quantitative evaluation of the content of human DNA in epithelial swabs
- Essential to improve the reliability of PCR study results.
- It can be used to quantify the content of the pathogen in a sample.
- Urogenital smears should contain enough epithelial cells.

RealBest® Technology: Solution for PCR diagnostics of sexually transmitted infections



Specimen collection | Nucleic acids extraction | Amplification | Interpretation of results | Export of analysis results

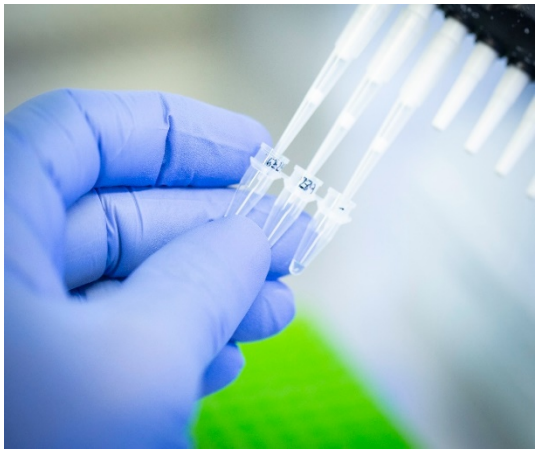
Nucleic acid isolation possible in both automatic and manual modes

Express – isolation

- the fastest and easiest
- ideal for screening

Sorption on magnetic particles

- a wide range of biological materials
- effective purification of nucleic acids



Kits "RealBest®" for the diagnosis of sexually transmitted infections

- **Ready Master Mix for PCR – ready to use**: just add isolated NA to the test tube and start the reaction
- **Multiplexity:** detection of two or more infectious markers in one tube
- **Universal protocol**
- **Possibility of automation:** improvement of quality and quantity of tests
- **Easy to store and transport**: no freezing required

Cat. №	Kit name	Number of tests
	Extraction kits for the isolation of nucleic acids	
8899	**RealBest DNA - express**	100
8878	**RealBest DNA-extraction 3 (variant 2x48)** (for KingFisher Flex and TECAN Freedom EVO)	96 (2x48)
	Validation of biological specimen sampling	
8888 CE	**RealBest Sample Validation**	96
	Kits for STDs detection	
1998	**RealBest DNA Chlamydia trachomatis**	96
2098 CE	**RealBest DNA Trichomonas vaginalis**	96
4396 CE	**RealBest DNA Mycoplasma genitalium**	96
4498 CE	**RealBest DNA Neisseria gonorrhoeae**	96
4494 CE	**RealBest DNA Neisseria gonorrhoeae (test 2)**	96
0455	**RealBest DNA Chlamydia trachomatis/Ureaplasma species**	96
0492	**RealBest DNA Chlamydia trachomatis/Ureaplasma urealyticum**	96
0490	**RealBest DNA Chlamydia trachomatis/Mycoplasma genitalium**	96
0498	**RealBest DNA Chlamydia trachomatis/Trichomonas vaginalis**	96
0457	**RealBest DNA Chlamydia trachomatis/Neisseria gonorrhoeae**	96
0494 CE	**RealBest DNA Mycoplasma hominis/Mycoplasma genitalium**	96
0496 CE	**RealBest DNA Trichomonas vaginalis/Neisseria gonorrhoeae**	96
0477 CE	**RealBest DNA Trichomonas vaginalis/Gardnerella vaginalis**	96
0444 CE	**RealBest DNA Candida albicans/Gardnerella vaginalis**	96
0488	**RealBest PCR-12 STI** *Chlamydia trachomatis*, *Ureaplasma species*, *Mycoplasma hominis*, *Mycoplasma genitalium*, *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, *Candida albicans*, *Gardnerella vaginalis*, cytomegalovirus, herpes simplex virus 1 a 2, HPV 16 and 18 (differential diagnosis)	96

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