DNA-TECHNOLOGY

Labcare de Colombia

CATALOG PCR KITS

Moscow
2018

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NOTE! **The information contained in this catalog may not be consistent with the latest version of the specifications for the indicated product**

ABBREVIATIONS

AASLD American Association for the Study of Liver Diseases
ABCB1 ATP-binding cassette, sub-family B
ACS Acute Coronary Syndrome
ADP Adenosine Diphosphate
AIDS Acquired Immune Deficiency Syndrome
ARVI Acute Respiratory Viral Infections
ASA Acetylsalicylic Acid
BGC Bacterial Genome Count
BMD Bone Mineral Density
cDNA Complementary DNA
CFTR Cystic Fibrosis Transmembrane conductance Regulator
CHEK2 Cell cycle heckpoint kinase 2
CHC Chronic Hepatitis C disease
CMV Cytomegalovirus
CNS Central Nervous System
COL1A1 Collagen, type I, alpha 1
COPD Chronic Obstructive Pulmonary Disease
CTR Calcitonin Receptor
CVD Cardiovascular Disease
DBP Vitamin D-binding Protein
DNA Deoxyribonucleic Acid
dATP deoxyadenosine triphosphate
dGTP deoxyguanosine triphosphate
dNTP deoxynucleotide triphosphate
dTTP deoxythymidine triphosphate
dCTP deoxycytidine triphosphate
EAA European Academy of Andrology
EASL European Association for the Study of the Liver
EBV Epstein-Barr virus
EDIs Especially Dangerous infections
ER Estrogen Receptor
ESR1 Estrogen Receptor1
FDA Food and Drug Administration – (a US federal agency responsible for protecting and promoting public health through regulation and supervision of food safety, pharmaceutical drugs and medical devices.)
FLASH Fluorescent Amplification-based Specific Hybridization
GBS Group B Streptococcus
GE Genome Equivalent
GMF Genetically Modified Food
GMO Genetically Modified Organism
GR Glucocorticoid Receptor
HBV Hepatitis B Virus
HCV Hepatitis C Virus
HER Human Epidermal Growth Factor Receptor 2
HHC Hereditary Hemochromatosis
HHV Human Herpes Virus
HIV Human Immunodeficiency
HLA human leucocyte antigens
HPV Human Papillomavirus
HSV Herpes Simplex Virus
ICD International Classification of Diseases
ICSI Intracytoplasmic Sperm Injection
IHD Ischemic Heart Disease
IHR International Health Regulations
IL Interleukin
INR International Normalized Ratio
IRT Immunoreactive Trypsin
IU International Unit
IVD *In vitro* diagnostics
IVF *In vitro* fertilization
MESA Microsurgical Epididymal Sperm Aspiration
NIBSC The National Institute for Biological Standards and Control
PBM Peak Bone Mass
PCR Polymerase Chain Reaction
PESA Percutaneous epididymal sperm aspiration
PID Pelvic Inflammatory Diseases
PII Percutaneous Intracoronary Intervention
RNA Ribonucleic Acid
PR Progesterone Receptor
PTH Parathyroid Hormone Receptors
qPCR Quantitative real time polymerase chain reaction
RANKL Receptor Activator of Nuclear Factor kappa-B Ligand
RAS Renin-Angiotensin System
RUO Research Use Only
RT-PCR Real-Time Polymerase Chain Reaction
RT Reverse Transcriptase
Rt Real Time
SARS Severe Acute Respiratory Syndrome
SCO Sertoli Cell-only Syndrome
SIC Sample Intake Control
SNP Single Nucleotide Polymorphism
SoGAT Standardization of Genome Amplification Techniques
SpA Seronegative Spondyloarthritis
STIs Sexually Transmitted Infections
STS Sequence-Tagged Site (short unique DNA sequences that can be amplified in the presence of other genomic DNA sequences)
SVR Sustained Virologic Response
TESA Testicular Sperm Aspiration
TESE Testicular Sperm Extraction
VDR Vitamin D Receptor
VZV Varicella Zoster Virus
WHO World Health Organization

THE TERMS USED

Allele (*from the Greek word 'allelon', meaning "of each other"*) refers to different forms of the same gene located in the same place (*locus*) on homologous chromosomes, which controls alternative variants of the same sign. All the genes of somatic cells, except for the genes located in the sex chromosomes, are represented by two alleles, one inherited from the father, and the other from the mother.

Gene (*from the Greek word 'genos', meaning birth, origin, race, species, or class*) is the material carrier of genetic information, which is a section of DNA, carrying the complete information about the structure and characteristics of the synthesis of one protein molecule.

Genetic polymorphism is the coexistence within a population of two or more different inherited forms for whose gene portion in a population there is more than one variant of nucleotide sequence. The most common is *single-nucleotide polymorphism (SNP)* – substitution of one nucleotide for another at a particular point of the genome.

Genotype *(from Greek words 'genos', which means birth or origin and 'typos' which means a mark)* is a set of alleles of a gene or group of genes controlling the analyzed traits in a given organism.

Heterozygous genotype (heterozygous state of a gene) is a genotype containing different alleles of one gene.

Hyperergia *(from Greek words 'hyper' meaning over, and 'ergon' meaning work)* refers to increased reactivity.

Homozygous genotype (homozygous gene) is a genotype containing identical alleles of one gene.

Multifactorial diseases (diseases with hereditary component) are diseases, which develop from interaction of certain genetic factors and specific impacts from environmental factors.

Nucleotide is a complex chemical group found in a natural state. It consists of a nitrogenous base, linked to a sugar, and phosphoric acid. It is single unit of nucleic acid (DNA and RNA) molecule. There are four types of nucleotides that make up the nucleotide sequence: **A** (adenine), **G** (guanine), **T** (thymine), **C** (cytosine) – the DNA sequence; **A** (adenine), **G** (guanine), **T** (thymine), **U** (uracil – not found in DNA; thymine is replaced by uracil in RNA) – the RNA sequence.

Positive predictive value is the probability of having a disease with a positive (pathological) test result.

Risk factors – the general name of factors that are not the direct cause of a specific disease, but make a person more likely to develop the disease. They are divided into modifiable (behavioral) and non-modifiable (physiological) risk factors.

Phenotype *(from Greek words 'phainon', which means revealing and 'typos', which means mark)* designates the totality of manifestations of a genotype (the overall appearance of the body), and in the narrow sense – an individual's observable traits (phenes), controlled by certain genes. The concept 'phenotype' applies to any traits of the body, starting from the primary products of gene action (RNA and polypeptide molecules) and ending with characteristics of the external structure, physiological processes, behavior, etc. The phenotype is formed based on interaction of the genotype and a number of environmental factors.

OR (odds ratio) is the ratio of chances. It is defined as the chances of an outcome under the influence of a risk factor divided by the chances of that outcome without the influence of a risk factor. In this case, it is used to estimate the chances of developing a clinical condition depending on the genotype of an individual. OR> 1 corresponds to an increased risk of developing the condition being analyzed, while OR<1 corresponds to a decrease in the risk.

ABOUT THE COMPANY

DNA-Technology is a unique company for Russian biotech business environment due to its full-cycle R&D and manufacturing process.

The company has been developing, producing and introducing to laboratories high-tech equipment and kits for PCR assays since 1993.

Our team brings together leading experts in the field of molecular biology, immunogenetics, medicine, thermodynamics, optics, electronics and programming. Implementing skills they form technological and scientific potential of the company, that allows providing a high standards and quality control of production at all stages.

DNA-Technology production sites meet all the modern requirements for medical equipment and PCR kits production. This is evidenced by the license issued by the Federal Service on Surveillance in Healthcare and Social Development of Russian Federation and the certificates verifying the fact that the quality management system is complied with ISO 9001:2015 and ISO 13485:2016.

THE MAIN DIRECTIONS OF «DNA-TECHNOLOGY» ACTIVITIES:

- Customers support at any level of PCR laboratory workflow: designing a plan for laboratory facilities, supplying kits, equipment and consumables, personnel training, analyzing the results of genetic tests and interpreting them for clinicians.
- Developing and manufacturing of equipment and software for PCR analysis in the scientific and clinical fields.
- Producing a wide range of kits for clinical bacteriology, virology, genetic diagnostic and detection of DNA of infectious agents in agricultural crops.
- Providing service support.
- Joint projects with clinicians and researchers.

Our product range includes basic equipment and devices for PCR laboratories:

- Detecting thermocyclers for real-time PCR analysis (*DT* devices);
- Thermostats;
- Power supplies;
- PCR cabinets.

The company has a strong R&D department for developing high-sensitivity kits for PCR analysis, such as:

- detection of viral and bacterial infections:
  - Hepatitis and HIV;
  - Urogenital infections;
  - Herpesvirus infections;
  - Human papillomavirus infections;
  - Respiratory tract infections;
  - Especially dangerous and natural focal infections;
  - Other infections;
- Analysis of microbiome composition of the urogenital tract;
- Molecular Genetics.

The company provides unique technologies for diagnostic of genetic predisposition to disorders such as polyorganic pathologies, oncology diseases, disorders of metabolic processes, malfunctioning of immune system and more.

Our goal is to apply the latest scientific findings in the field of molecular biology methods to routine laboratory practice. We do our best to improve diagnostic quality in order to make the treatment more effective and the prognosis of clinical outcome more precise.

I. PCR ADVANTAGES

PCR method is one of the most rapidly developing field in molecular genetic analysis. Extensive use of PCR technology has made it possible to significantly improve the quality of laboratory analysis and to reduce time of analysis.

Nowadays a modern PCR laboratory provides an opportunity to carry out multifactorial, multiplex and quantitative analysis by detecting nucleic acids of various microorganisms in the given sample.

Real-time PCR method makes it possible to detect the presence of an infectious agent (qualitative analysis) and DNA/cDNA concentration in the sample (quantitative analysis) in the diagnosis of infectious diseases.

Qualitative analysis is used to detect non-opportunistic pathogens, such as gonorrhea, chlamydia and trichomonas.

However, the situation for opportunistic pathogens is quite different – qualitative analysis is not sufficient for this group of microorganisms, as the term "opportunistic" itself implies that the only fact of their presence does not mean the presence of a disease. And it is important not only to detect the presence of the microorganisms in a sample, but their concentration as well. In this case, it is reasonable to apply quantitative real-time PCR analysis. Moreover, quantitative analysis is recommended for determining viral load and treatment for viral hepatitis B and C, and HIV.

It is important to note that in some cases it is vital to make a diagnosis within a short period of time with high degree of accuracy in order to avoid development of complications. Applying conventional methods of diagnosis such as culture and microscopic assays do not ensure proper diagnosis and high reliability due to objective reasons.

For example, the main objectives of microscopy are:

- Detection of a causative agent in the clinical material;
- Tentative identification based on the detection of morphological and tinctorial marks of microorganisms;
- Analysis of pure culture.

This method is considered as the fastest and cheapest and its use is associated with minimum requirements for laboratory's establishment.

However, there are a number of limitations in using microscopy for infectious diseases diagnosis:

- Low sensitivity;
- Subjectivity of the results assessment;
- Limited range of the morphotypes detected;
- Approximate quantitative assessment.

The culture method, alongside with microscopic examination of microorganisms, is included in the "golden standard" for diagnostics and allows:

- To detect all living cultivated organisms;
- To determine the antibiotic resistance of microorganisms detected.

However, the culture method has objective limitations:

- Long cultivation time – from 5 days to 2 months;
- More stringent requirements for biomaterial transportation and storage ;
- Inability to culture most of anaerobic microorganisms;
- More stringent requirements for laboratory's establishment.

Introducing PCR methods to laboratory practice provides modern laboratories with certain important advantages, such as:

- **High-speed analysis** – even the most complex multi-parameter assays take less than one day from the moment the sample is collected to the moment the results are obtained;
- **Determination of microorganisms concentration in a biomaterial;**
- **High reproducibility of results;**
- **Maximum sensitivity and specificity;**
- **Standardized technological process;**
- There is an opportunity for a wide range of infectious agents to be diagnosed from only one sample of biomaterial (when applying other methods it may be required to collect a sample from a patient several times, which may be traumatic and that's why unwelcome);
- Results can be obtained irrespective of the infectious process;
- A patient can be examined regardless of antibiotics treatment;
- Determining genetic factors of microorganisms' antibiotic resistance (options for the most effective therapeutic regimen to be selected).

The important aspect of PCR diagnostics is possibility to conduct genetic analysis, as well as to determine risks of somatic pathologies behaviour (such as thrombophilia) and drug tolerance.

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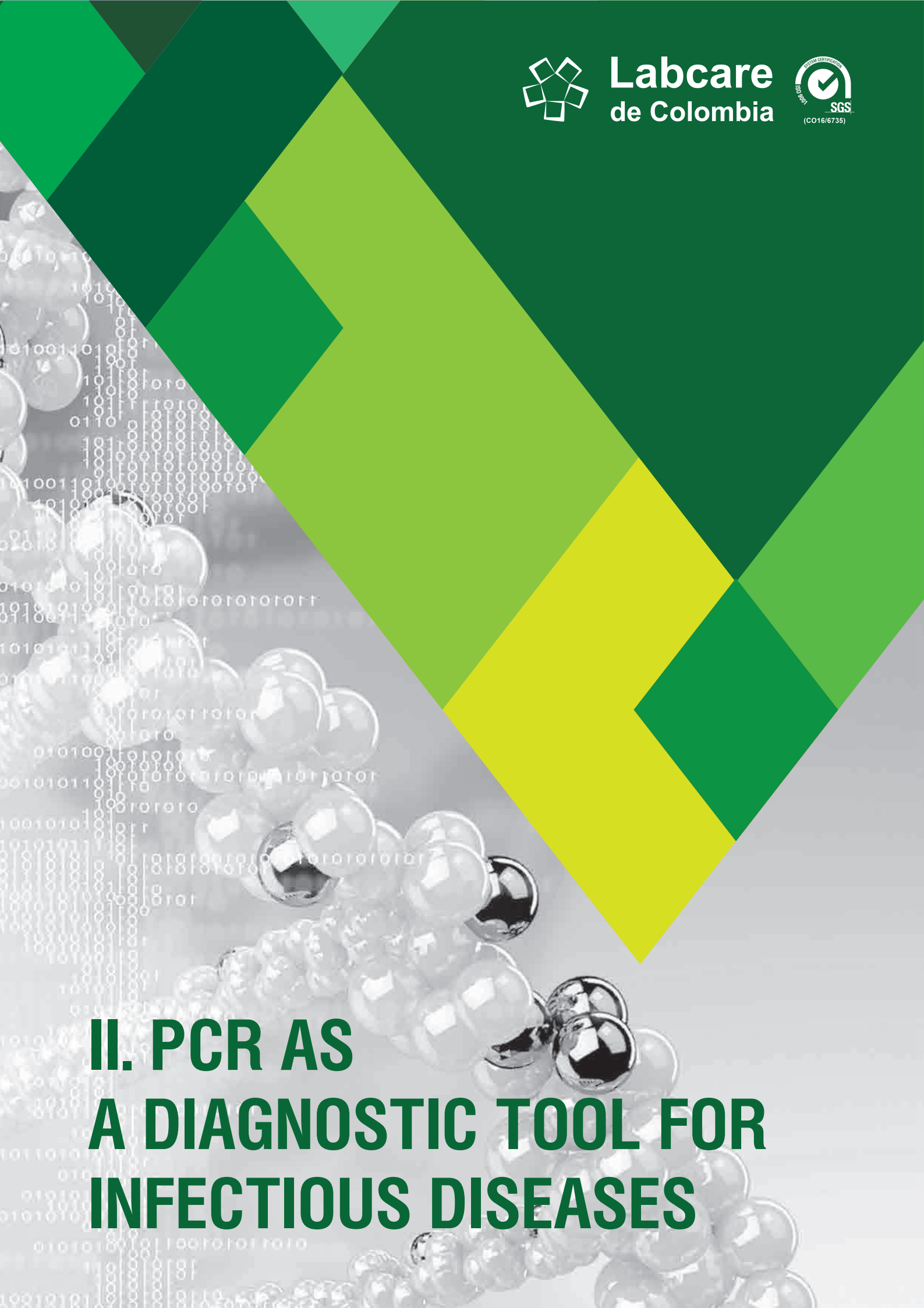
SGS (CO16/6735)



II. PCR AS A DIAGNOSTIC TOOL FOR INFECTIOUS DISEASES

II. PCR AS A DIAGNOSTIC TOOL FOR INFECTIOUS DISEASES

1. OPPORTUNISTIC AND NON-OPPORTUNISTIC PATHOGENS OF THE HUMAN UROGENITAL TRACT

The main causative agents of urogenital infections (sexually transmitted infections) are: *Treponema pallidum, Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis and Mycoplasma genitallium*. The role of these infectious agents in the development of urogenital tract diseases has been proven and now their diagnosis via qualitative methods is beyond question.

Mycoplasma hominis, Ureaplasma urealyticum and *Ureaplasma parvum* can be present in the urogenital tract together with non-opportunistic pathogens. These microorganisms are opportunistic organisms capable of causing a disease when exposed to a variety of endogenous and exogenous factors. At present the role of these microorganisms in disease development is controversial and requires further clarification.

Experts from the World Health Organization (WHO) in 2006 identified *U.urealyticum* as a potential pathogen of non-specific nongonococcal urethritis in men and possibly of pelvic inflammatory diseases (PID) in women. Experts from the US Centers for Disease Control and Prevention (CDC), 2010) do not accept the etiological role and clinical significance of genital mycoplasmas (except for *M.genitalium*) as established.

At the present stage, urogenital tract infections do not have pathognomonic symptoms and they proceed with latent, very mild clinical manifestations, with prevalence of the disease chronic forms. This results in complications such as pelvic inflammatory diseases, reproductive disorders, pregnancy complications, etc..

In this regard, *molecular biological techniques*, primarily PCR method, are becoming the most relevant in STIs diagnostics.

PCR method enables to:

- Identify the etiology of the infection;
- Maintain control over the infection;
- Assess the relative (compared with normal flora) amount of microorganisms, which is important for opportunistic pathogens that cause pathology only under certain conditions (increased concentration due to lower amount of normal flora);
- Evaluate treatment effectiveness.

Among all the undeniable merits of PCR in diagnostics of infectious agents, two of them stand out: ability to detect etiologic agents of the disease in introduction of mixed infection (conducting multiplex analysis) and low traumatism rate when sampling biomaterial is taken for examination.

The high sensitivity and specificity of PCR makes this technique appropriate for STIs diagnosis. In accordance with CDC Guidelines, European guideline for the management of Chlamydia trachomatis infections, and Treatment Guidelines for sexually transmitted diseases, only PCR-based diagnostics can be recommended for *C. trachomatis* detection.

Subjects of screening for STIs are:

- Persons that had sexual contact with STIs patients;
- Persons who are undergoing screening for other STIs;
- Persons from decreed group – during mandatory (at employment) and periodic medical examinations in accordance with approved regulations;
- Women with muco-purulent cervical discharge and adnexitis symptoms;
- Newborns from mothers who have had STIs during pregnancy;
- Sexual partners undergoing preconception checkup;
- Pregnant women;
- Women awaiting pelvic surgery;
- Sexually abused persons;
- Men with mucopurulent urethral discharge and dysuria symptoms;
- Egg and sperm donors;
- Persons under age of 25, who have numerous sex partners;
- Women with infertility, recurrent miscarriage and premature birth history.

Technique for collecting clinical specimen for PCR method:

- Epithelial cell scrapings are used as the specimen.
- The clinical material is obtained using a disposable sterile instrument, such as Cytobrush or scrape for DNA analysis. ***Use of brushes during pregnancy is contraindicated!***
- Before obtaining clinical specimen, freely flowing discharge is removed with a sterile cotton swab.
- Epithelial cell scrapings are obtained by rotating the probe.
- The obtained specimen is put into a tube, such as Eppendorf tube, that contains transport medium.
- The tube with the biomaterial is stored and transported at a household refrigerator temperature (+4 °C) without freezing, for not longer than 1 day.

Clinical specimen may be collected from the following potential infection sites:

Urethra:

Before obtaining the biomaterial, it is recommended to delay urination for at least 1.5 hours;

To obtain a clinical specimen from the urethra, a swab is inserted into male 2-4 cm deep and into female – 1.5-2 cm;

In case of abundant purulent discharges, the clinical specimen should be obtained after urination.

Vagina: a clinical specimen is obtained from the posterior or posterolateral vaginal wall

Endocervical canal: to obtain a clinical specimen, the swab is inserted up to 1.5-2 cm deep.

Rectal ampulla: a clinical specimen is obtained by moving the swab in a circular motion from all over the walls. The swab is inserted up to 3-4 cm deep.

Conjunctiva: a clinical specimen is obtained from the surface of the inferior eyelid conjunctiva moving from the outer to the inner corner of the eye.

Nasopharynx: a clinical material is obtained from posterior pharyngeal wall above the bottom edge of the soft palate and from the surface of the tonsils.

DNA-Technology offers the following kits (see Table 1) for detecting urogenital infections by PCR method.

Table 1. Kits produced by DNA-Technology for detecting urogenital infections

Etiologic agent	Detection format				Registration*
	Forez	Flash	Rt	qPCR	
Chlamydia trachomatis	*	*	*	–	RU/IVD
Mycoplasma hominis	*	*	*	–	CE/IVD
Mycoplasma genitalium	*	*	*	–	CE/IVD
Ureaplasma complex (U.urealyticum/ U.parvum)	*	*	*	–	CE/IVD
Ureaplasma parvum	*	*	*	–	CE/IVD
Ureaplasma urealyticum	*	*	*	–	CE/IVD
Trichomonas vaginalis	*	*	*	–	CE/IVD
Gardnerella vaginalis	*	*	*	–	CE/IVD
Neisseria gonorrhoeae	*	*	*	–	CE/IVD
Candida albicans	*	*	*	–	CE/IVD
TNC multiplex *(T.vaginalis/ N.gonorrhoeae/ C.trachomatis)*	–	–	*	–	RU/IVD
UMC multiplex *(U.urealyticum; parvum/ M.genitalium/ C.trachomatis)*	–	–	*	–	RUO

*** Note:**

RU/IVD – kits for *In Vitro* Diagnostic, which are registered in Russia only

CE/IVD – kits for *In Vitro* Diagnostic, which are registered in EU

RUO – kits for Research Use Only

Kit format:
- Strip tubes (8 pcs., 0.2 ml each);
- Single tubes (0.5 ml or 0.2 ml).

Storage temperature: from +2 to +8 °C.

Shelf life:
- Forez – 9 months;
- FLASH – 12 months;
- Rt – 12 months (except *TNC multiplex* and *UMC multiplex* kits – 6 months).

DNA extraction kits of reagents:
- *PREP-RAPID;*
- *PREP-NA;*
- *PREP-GS.*

Specimen for PCR testing:
- Scrapings from mucosa ;
- Urine cell sediment.

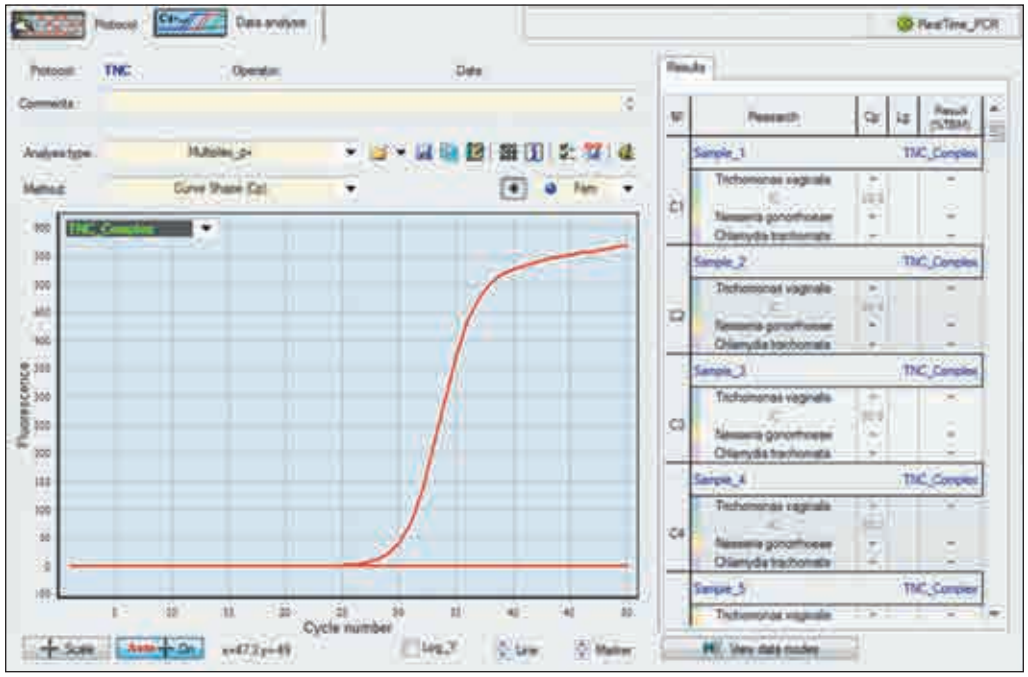
Recommended additional reagents:
SIC – sample intake control – is designed for DNA identification (qualitative analysis) and approximate estimation of the amount of human genomic DNA (quantitative analysis) in a human biomaterial by real-time PCR.

Equipment required for analysis:
- For FLASH kits: Gene, Gene-4 or counterparts
- For Rt kits:
  - DT devices produced by DNA-Technology (DTlite, DTprime, DT-96);
  - IQ5 Cycler device produced by Bio-Rad Laboratories and Rotor-Gene devices produced by QIAGEN (except for *TNC multiplex* and *UMC multiplex* kits).

The following additional equipment is needed for analysis using strip tubes:
Strip plastic rack and centrifuge (vortex) rotor.

Software: Reaction results are analyzed and interpreted automatically (for devices produced by DNA-Technology) (Fig. 1).

A



B

№	Name of research	Results
1	Trichomonas vaginalis	not discovered
2	Neisseria gonorrhoeae	not discovered
3	Chlamydia trachomatis	+

Fig. 1. Results of *Rt* qualitative analysis (DT devices) TNC Multiplex REAL-TIME PCR Detection Kit
A – Optical measurement analysis (Fam channel)
B – Analysis results

2. HERPESVIRUS INFECTIONS

Herpesviruses are abundant in nature. Nowadays more than 100 herpes viruses are distinguished, a small amount of which occurs in humans.

Herpesvirus can remain persistent in a human body for the entire lifetime causing diseases with multiple clinical implications (see Table 2).

Table 2. Diseases caused by herpesviruses

Type of herpesvirus	Diseases caused
Labial herpes simplex virus (type 1)	Herpes simplex: lesions of skin, mouth mucous membrane, conjunctiva or cornea, encephalitis
Genital herpes simplex virus (type 2)	Genital herpes: lesions of genitals mucous membranes, central nervous system damage in newborns
Varicella zoster virus (type 3)	Chickenpox, herpes zoster, postherpetic neuralgia, necrotizing herpes zoster in HIV infection
Epstein-Barr virus (type 4)	Infectious mononucleosis, Burkitt's lymphoma, nasopharyngeal cancer, genital ulceration
Cytomegalovirus (type 5)	Cytomegalovirus infection: neonatal pathologies, transplantation complications, such as interstitial pneumonia, gastrointestinal disorders, hepatitis
Herpesvirus (type 6)	Infectious roseola (exanthem subitum), interstitial pneumonia
Herpesvirus (type 7)	Chronic fatigue syndrome, exanthem subitum
Herpesvirus (type 8)	Kaposi sarcoma

Herpes simplex virus (HSV), Type 1 and 2.

Herpes simplex virus invades a human body through damaged skin and mucous membranes. During the first phase of pathogenesis, the virus gets into epithelial cells (mucosa of oral cavity, pharynx or genital organs), where its replication occurs. Papules and vesicles, characteristic for herpes infection, appear on mucosa and skin.

Genital herpes (GH) is a herpes simplex virus. It remains persistent in men in the urogenital tract, and in women in the cervical canal, vagina and urethra. Infecting with genital herpes occurs mainly through sexual contact. During viremia in pregnant women, a fetus is infected via hematogenic route, and during childbirth via contact. Herpes infection arising from viremia may result in afflicting several organs at a time.

Herpetic esophagitis, pneumonitis and hepatitis can develop. Damage to peripheral nervous system can occur in the form of ganglionitis, ganglionevrita, radiculoneuritis and polyneuropathy. Central nervous system (CNS) damage with herpes infection most commonly proceeds as encephalitis or meningoencephalitis.

Specimen for PCR testing:

- Vesicles fluid;
- Scrapings from mucosa ;
- Blood;
- Mucus;
- Urine;
- Lacrimal fluid;
- Cerebrospinal fluid.

PCR specimen is taken from eruption sites only during the period of acute infection.

Cytomegalovirus (CMV)

The virus invades a body through saliva, genital secretions during sexual intercourse, through breast milk, during organ transplantation and donor blood transfusion, when using donor sperm and ovigerms, as well as non-sterile syringes. Moreover, introduction of cytomegalovirus (CMV) infection can occur even during fetus prenatal development period in pregnant women with viremia.

CMV infection mostly manifests itself as:

- ARVI (acute respiratory viral infection). Complaints of weakness, general malaise, rapid fatigability, headache, runny nose, sialadenosis, saliva flux and whitish film on gums and a tongue.
- Generalized CMV infection with visceral injury, inflammatory disorder of:
  - Hepatic tissue;
  - Adrenal glands;
  - Spleen;
  - Pancreatic glands;
  - Kidney.

This is accompanied by frequent pneumonia and bronchitis that poorly respond to antibiotic therapy. Lesions of eye vessels, intestinal wall, brain and peripheral nerves, as well as enlarged parotid and submandibular salivary glands, arthritis, and skin rash are rather common.

- Lesion of urogenital organs in men and women manifests itself by symptoms of chronic non-specific inflammation.
- Pathologies in pregnancy, fetus and newborn result in progress of serious illness and central nervous system damage (mental developmental delay, hearing loss). In 20-30 % of the cases a baby dies.

Specimen for PCR testing:

- Urethral scrapings;
- Cervical and vaginal scrapings;
- Cell urocheras;
- Blood;
- Peripheral blood mononuclear cells;
- Cerebrospinal fluid.

Varicella Zoster Virus (VZV, HHV-3)

VZV causes two types of lesions: chickenpox and herpes zoster. The main infection transmission routes are airborne and non-percutaneous channels of infection (through discharge of vesicular fluid).

VZV is a causative agent of chicken pox in children. It invades a body during the childhood and remains in sensory cerebrospinal and cerebral ganglions in a latent form. With immunity deterioration the virus becomes active and causes radiculitis. In some cases retrograde spread of the virus in the CNS via sensitive pathways occurs.

Vesicular rashes appear on the skin of patients suffering from herpes zoster. Vasculitis, mostly observed after eye-lesion, can indicate that the virus has invaded CNS. VZV often affects HIV-infected people at different stages of the disease. Apart from herpes zoster, it can cause damage to the nervous system, such as encephalitis and myelitis.

Specimen for PCR testing:

- Vesicles fluid or rash scrapings;
- Scrapings from mucosa;
- Blood;
- Cerebrospinal fluid.

Epstein-Barr Virus (EBV, HHV-4)

Human is the only reservoir of infection. The virus is mainly transmitted by air; less frequently it is vector-borne or through sexual intercourse.

At an early age, the infection is accompanied by slight manifestations or it is generally asymptomatic; initial infection at teenage or more advanced age can cause a disease known as infectious mononucleosis. Chronic EBV infection is more common among patients with immunodeficiency. Chronic infection mostly manifests itself in the form of progressive lymphoproliferative disease or CNS lymphoma.

The virus can cause malignant transformation of cells, that gives reason to assume that it is involved in such diseases as African forms of Burkitt's lymphoma, nasopharyngeal carcinoma in men from some ethnic groups of southern China, as well as Kaposi sarcoma in patients with AIDS.

Specimen for PCR testing:

- Urethral scrapings;
- Blood;
- Peripheral blood mononuclear cells;
- Cerebrospinal fluid.

Herpes Virus Type 6 (HHV-6)

The disease is typically latent in nature, sometimes with clinical implications in the form of sudden exanthema (exanthema subitum, children roseola (sudden) or false rubella – an acute viral infection in young children), a syndrome similar to mononucleosis.

This virus is associated with chronic fatigue syndrome, which manifests itself as an incipience of a respiratory disease, with catarrhal symptoms, fever, sore throat, erratic myalgia, cervical, neck and axillary lymph-node hyperplasia, joint pain, sleep disturbances, muscle weakness, undue fatiguability, and hyperirritability.

The diagnostic criteria of this syndrome include: chronic fatigue and performance decrement by more than 50 % within 6 months in the absence of other diseases (cancer, diseases of liver, kidney, heart, etc.) which cause similar symptoms.

The virus is also associated with B-cell lymphoma. In 80-90 % of cases of B-cell lymphomas, integrated DNA fragments, homological to the virus genome, are detected in the transformed cell, which gives ground to consider it a potential etiologic agent of the disease.

Specimen for PCR testing:

- Blood;
- Urine;
- Phlegm;
- Throat swab.

Human Herpesvirus 8 (HHV-8)

It was found that HHV-8 is associated with all types of Kaposi sarcoma, including endemic African Kaposi sarcoma, classic Mediterranean Kaposi sarcoma among elderly people and transplant-related Kaposi sarcoma. The DNA of this virus is constantly detected in the tissue of AIDS-related Kaposi sarcoma, whereas it is not detected in the normal tissue of neighboring sections.

It was demonstrated that the course of Kaposi sarcoma is preceded by an infection caused by HHV-8. Moreover, the virus is a developing factor for primary lymphoma of serous sac, and some varieties of Castleman's disease.

Specimen for PCR testing:

- Blood;
- Urine;
- Phlegm;
- Throat swab.

DNA-Technology offers the following kits (see Table 3) for detecting herpesvirus infections by PCR.

Table 3. Kits produced by DNA-Technology for detecting herpesvirus infections

Etiologic agent	Detection format				Registration*
	Forez	Flash	Rt	qPCR	
Herpes symplex virus 1,2	*	*	*	–	CE/IVD
Human herpesvirus 6	*	*	*	–	RU/IVD
Human herpesvirus 8	*	*	*	–	RU/IVD
Cytomegalovirus	*	*	*	–	RU/IVD
Epstein Barr virus	*	*	*	–	CE/IVD
Varicella zoster virus	*	*	*	–	CE/IVD
Herpes multiplex (HSV1/HSV2/CMV)	–	–	*	–	RU/IVD

*** Note:**

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RUO – kits for Research Use Only

Kit format:

- Strip tubes (8 pcs., 0.2 ml each);
- Single tubes (0.5 ml or 0.2 ml).

Storage temperature: from +2 to +8 °C.

Shelf life:

- Forez – 9 months;
- FLASH – 12 months;
- Rt – 12 months (except for Herpes multiplex reagent kits – 6 months).

DNA extraction kits:
- *PREP-RAPID;*
- *PREP-NA;*
- *PREP-GS.*

Specimen for screening:
- Vesicles fluid;
- Scrapings from mucosa;
- Biopsy specimens;
- Blood;
- Cerebrospinal fluid.

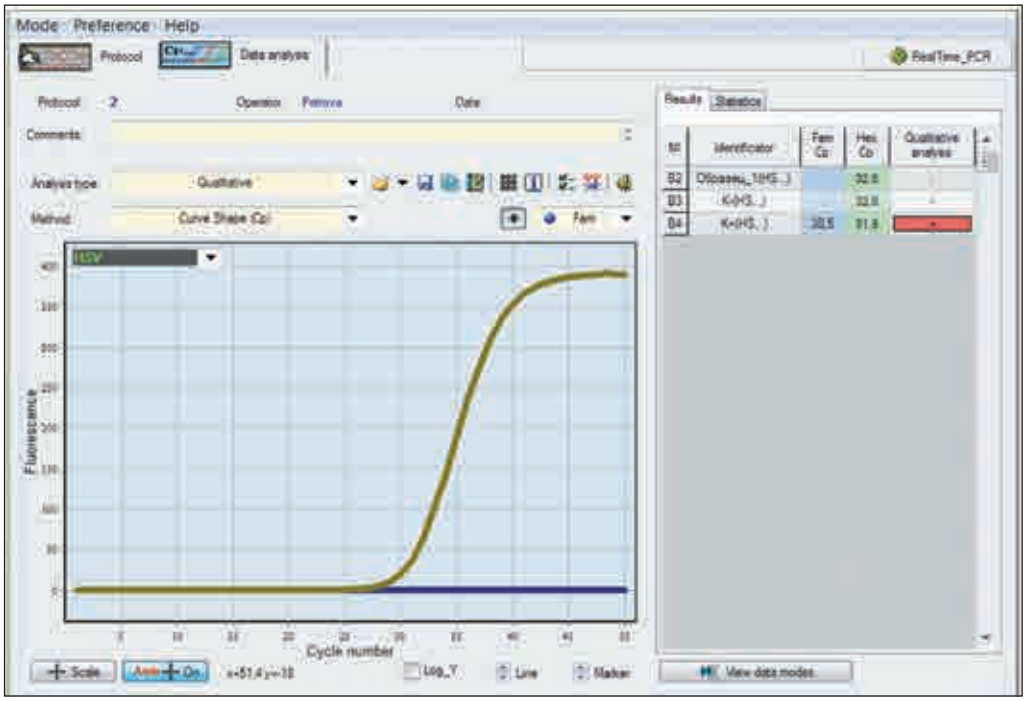
Recommended additional reagents:
SIC – sample intake control – is designed for DNA identification (qualitative analysis) and approximate estimation of the amount of human genomic DNA (quantitative analysis) in a human biomaterial by real-time PCR.

Equipment required for analysis:
- For *FLASH* kits: Gene, Gene-4 or counterparts
- For *Rt* kits:
  – DT devices produced by DNA-Technology (DTlite, DTprime, DT-96);
  – IQ5 Cycler device produced by Bio-Rad Laboratories and Rotor-Gene devices produced by QIAGEN (except reagent kit *Herpes multiplex*).

The following additional equipment is needed for analysis using strip tubes:
Strip plastic rack and centrifuge (vortex) rotor.

Software: Reaction results are analyzed and interpreted automatically (for devices produced by DNA-Technology) (Fig. 2).

A



B Qualitative analysis

Number of the hole	Identificator of the tube	Cp, Fam	Cp, Hex	Result
B2	Sample_456		32.6	–
B3	K–		32.6	–
B4	K+	30.5	31.9	+

Fig. 2. Results of *Rt* qualitative analysis (DT devices) HSV 1,2 REAL-TIME PCR Detection Kit
A – Optical measurement analysis (Fam channel)
B – Analysis results

3. HUMAN PAPILLOMAVIRUS INFECTIONS

Human papillomavirus (HPV) belongs to the genus of papilloma viruses of the Papovaviridae family and has a definitive biocycle associated with differentiation of keratinocytes. The virus infects only proliferating epithelial cells of the basal layer. Formation of viral particles occurs in the upper layers of the epithelium. Presently over 100 types of HPV are distinguished, with about 30 of them being able to infect the epithelium of a human urogenital tract.

Clinical implications of HPV infection:

- Skin lesions (plantar wart, common wart, flat wart, Butcher's wart, warty epidermodysplasia, Bowen's disease, non-warty skin lesions, and carcinoma).
- Lesions of the mucous genitals (*condylomata accuminata*, giant condyloma (Buschke-Lowenstein tumour), non-condylomatous lesions, carcinoma).
- Lesions of the mucous membranes of other organs (laryngeal papilloma, tonsillar carcinoma, neck carcinoma, tongue carcinoma).

Diseases associated with human papillomavirus infection:

- Anogenital (venereal) warts;
- Viral warts: simple, vulgar;
- Laryngeal papillomatosis;
- Benign neoplasms of male genital organs;
- Cervical neoplasia.

HPV infection route:

- Sexual way, including oral-genital contact and anal sex;
- Vertical transmission – at birth, that causes laryngeal papillomatosis with babies and anogenital warts with infants;
- Domestic path – external manifestations of the virus – pointed condyloma;
- Self-infection (autoinoculation).

Papillomaviruses are divided into two groups based on ability to induce malignant transformation of the epithelium: low-risk and high-risk oncogenic papillomas.

The low-risk oncogenic group includes Types 6, 11, 36, 42, 43, 44, 46, 47 and 50. An infection caused by these types usually occurs in the form of benign cervical lesions and condylomas.

The high-risk oncogenic group includes Types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73 and 82. Type 16 is the most common and it is detected in over 50 % of all cervical cancer cases in Europe.

Cervical cancer is the second largest (after breast cancer) malignant tumor of the reproductive system. In the age group from 16 to 40, cervical cancer is the second leading cause of mortality among patients with malignant tumors.

The disease progression from epithelial infection to cervical cancer formation takes at least 5 years (10-20 years on average), and only in very rare cases it can take 1-2 years. That is why a significant role in cervical cancer prevention should be given to screening that would detect the presence of HPV infection and precancerous changes or cancer at the early stages.

The most frequently used test for screening of cervical cancer and precancerous changes in the epithelium is a *cytological* one. At the same time the diagnostic accuracy can vary depending on the specimen collection method, binding technique and smear preparation as well as a researcher's skills. It is believed that nearly one third of cervical cancer cases are diagnosed in women who were screened regularly during cytological examination and thus false negative results for these patients were obtained. Therefore, DNA diagnostics of HPV infection is currently considered the basis for cervical cancer screening and prevention.

The fact of HPV presence can be used for adjusting an examination plan and frequency of monitoring of pacient. The positive test result has high diagnostic significance for women's age over 30. DNA diagnosis is used as a confirmatory test when detecting ASC-US (after liquid cytology and visual method) and when monitoring a therapy for CIN II, III.

DNA-Technology offers the following kits (see Table 4) for detecting papillomavirus infection and typing low-risk and high-risk oncogenic human papillomas by PCR.

Table 4. Kits produced by DNA-Technology for detecting human papillomavirus infection

Etiologic agent	Detection format				Registration*
	Forez	Flash	Rt	qPCR	
HPV 16,18 PCR detection Kit	*	*	*	–	RU/IVD
HPV 16/18 multiplex	–	–	*	–	RUO
HPV 16, 31, 33, 35, 35H, 58, 52, 67	*	–	–	–	RUO
HPV 18, 45, 39, 59	*	–	–	–	RUO
HPV 51,26	*	–	–	–	RUO
HPV 6, 11	*	–	–	–	RUO
HPV 6/11 multiplex	–	–	*	–	RUO
HPV 6	–	–	*	–	RUO
HPV 11	–	–	*	–	RUO
HPV QUANT-4 (HPV 16,18)	–	–	*	*	RU/IVD
HPV QUANT-4 (HPV 6,11,16,18)	–	–	*	*	CE/IVD
HPV QUANT-15 (HPV 16, 31, 33, 35, 52, 58, 6, 11, 18, 39, 45, 59, 51, 56, 68)	–	–	*	*	CE/IVD
HPV QUANT-21 (HPV 6, 11, 44, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73,82)	–	–	*	*	CE/IVD

*** Note:**

RU/IVD – kits for *In Vitro* Diagnostic, which are registered in Russia only

CE/IVD – kits for *In Vitro* Diagnostic, which are registered in EU

RUO – kits for Research Use Only

Kit format:

- Strip tubes (8 pcs., 0.2 ml each) (only strip tubes for kit HPV QUANT);
- Single tubes (0.5 ml or 0.2 ml).

Storage temperature: from +2 to +8 °C.

Shelf life:

- Forez – 9 months;
- FLASH – 12 months;
- Rt – 12 months (except HPV QUANT, HPV 6/11 multiplex and HPV 16/18 multiplex kits – 6 months).

DNA extraction kits:

- *PREP-RAPID;*
- *PREP-NA;*
- *PREP-GS;*
- *PREP-NA-PLUS (for use with HPV QUANT);*
- *PREP-GS-PLUS (for use with HPV QUANT).*

Specimen for screening:

Scrapings from mucosa of the urogenital tract.

Recommended additional reagents:

- SIC – sample intake control – is designed for DNA identification (qualitative analysis) and approximate estimation of the amount of human genomic DNA (quantitative analysis) in a human biomaterial by real-time PCR;
- For HPV QUANT, SIC is part of the kit.

Equipment required for analysis:

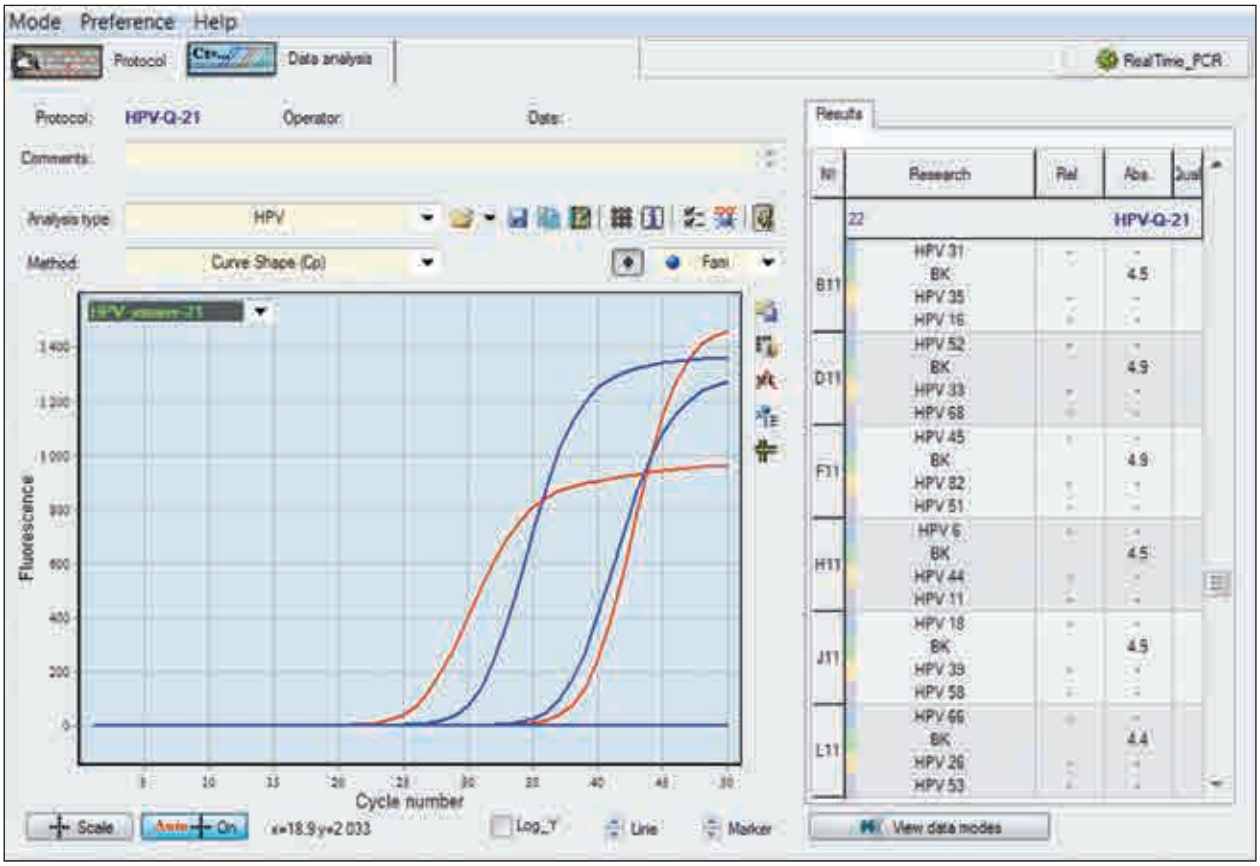
- For *FLASH* kits: Gene, Gene-4 or counterparts
- For *Rt* kits:
  - DT devices produced by DNA-Technology (DTlite, DTprime, DT-96);
  - IQ5 Cycler device produced by Bio-Rad Laboratories (except for HPV QUANT kits) and Rotor-Gene device produced by QIAGEN (only HPV 16,18)

The following additional equipment is needed for analysis using strip tubes:

Strip plastic rack and centrifuge (vortex) rotor.

Software: Reaction results are analyzed and interpreted automatically (for devices produced by DNA-Technology) (Fig. 3).

A



B

HPV

Date
Number of tube
Patient name
Sex
Age
Organization
Clinician name
Comments

Information about laboratory

Sample ID: 22

№	Name of research	Results		
		Relative, (X/SIC)	Quantitative, Lg (coples/sample)	Qualitative
1	HPV 31	not discovered	not discovered	
2	HPV 35	not discovered	not discovered	
3	HPV 16	not discovered	not discovered	
4	HPV 52	not discovered	not discovered	
5	HPV 33	not discovered	not discovered	
6	HPV 68	not discovered	not discovered	
7	HPV 45	not discovered	not discovered	
8	HPV 82	not discovered	not discovered	
9	HPV 51	not discovered	not discovered	
10	HPV 6	not discovered	not discovered	
11	HPV 44	not discovered	not discovered	
12	HPV 11	not discovered	not discovered	
13	HPV 18	not discovered	not discovered	
14	HPV 39	not discovered	not discovered	
15	HPV 58	not discovered	not discovered	
16	HPV 66	not discovered	not discovered	
17	HPV 26	not discovered	not discovered	
18	HPV 53	not discovered	not discovered	
19	HPV 59	not discovered	not discovered	
20	HPV 56	not discovered	not discovered	
21	HPV 73	not discovered	not discovered	
22	SIC		4.8	

Study was carried out

Date
Signature

Fig. 3. Analysis results for Rt optical measurements (DT devices)
The HPV-QUANT-21® *quantitative PCR Kit*
A – Optical measurement analysis (Fam channel)
B – Analysis results

4. HCV, HBV AND HIV VIRUSES

During diagnosis of *blood-borne infections*, PCR technology has great importance especially in blood banking. In 1995 on the recommendation of the National Institute for Biological Standards and Control, UK (NIBSC) under WHO a working group SoGAT was established to exchange experience in introducing gene testing for the most transfusion-dangerous viruses – HIV, hepatitis B, C, A viruses, parvovirus, and others for blood banking and other areas.

The experience of applying gene test proved the efficiency of this method in detecting donors- virus carriers, especially in seronegative period. Since July 1999, gene test has become mandatory in the EU countries for all donated blood and plasma. Currently, PCR technology solves the following blood banking tasks:

- Ensuring infection safety during the period of "seronegative window";
- Providing information that no infectious agents were found in the blood or blood components with ambiguous results of enzyme multiplied immunoassay obtained;
- Identifying true virus carriers among seropositive individuals.

In laboratory diagnosis of viral hepatitis and HIV infection, there is a number of problems that PCR method can resolve, thereby enabling a doctor to make an early diagnosis and commence treatment.

- Discrepancies between PCR results and enzyme-linked immunosorbent assay (ELISA) results – positive PCR result and negative ELISA result – are the most urgent challenges in detecting hepatitis C and human immunodeficiency viruses. This fact can be explained by the period of "serological window". Typically detectable amounts of HIV antibodies appear in the blood 2-10 weeks after infecting. However, variability of periods can be quite large. For example, HIV antibodies are detected within three months after infecting in 90-95 % of those infected, after six months in 5-9 % of patients, and at a later date in 0.5-1 %. Detection of DNA/RNA virus by PCR method reduces the duration of the period of "serological window" by 11 days on average and helps detect the pathogen within 1-2 weeks after infecting.
- With PCR it is possible to detect the RNA of hepatitis C virus not only in blood serum but also in liver biopsy specimens, which is important in confirming the role of hepatitis C virus in the formation of hepatocellular carcinoma. In such patients, hepatitis C virus RNA is detected in hepatocytes and in the absence of anti-HCV and HCV RNA in blood serum. Anti-HCV does not appear at all among a number of patients with self-limited course of infection.
- Occurrence of maternal antibodies in infants born by infected mothers can also distort the true picture. Since maternal IgG antibodies penetrate through the blood-placenta barrier, the antibody test result in babies born by such mothers will be positive for a long time (up to a year and a half after the birth). In this case, the presence or absence of the virus in the body of a baby can only be proven by using direct methods for detecting the virus, PCR being one of them.

Detecting the viral genotype is an important aspect that determines treatment management and further care of hepatitis C patients. This is explained by the fact that HCV is characterized by high variability and the presence of several variants of genotype. In clinical practice, it is important to distinguish 5 HCV subtypes: 1a, 1b, 2a, 2b, 3a.

So, among patients with subtype 1b, chronization of HCV infection occurs in 90 % of cases, while for genotype 2a and 3A patients, it occurs in 33-50 %. Genotype 1b infection is accompanied by more severe disease, development of liver cirrhosis and hepatocellular carcinoma. Patients with subtype 3a have franker steatosis and biliary tract lesions, as well as higher ALT levels as compared with the patients having HCV genotype 1b. At the same time fibrosis levels are more pronounced in patients with subtype 1b virus.

Determining the viral loads is the key factor in viral hepatitis and HIV treatment:

- Timing of the first therapy – for HIV infection, prescription of HAART depends on CD4+ concentration and viral load.
- Monitoring of the efficacy of antiviral therapy. For treatment of viral hepatitis and HIV infection, a range of pharmaceutical products has been developed whose effectiveness can be different for each individual patient. When selecting an adequate dosage scheme, it is required to conduct quantitative analysis of the virus content in the blood (viral load) by real-time PCR as the primary measure of efficiency.

Standardization of quantitative methods for determining DNA in the blood serum resulted in use of *International Units* (IUs). IUs do not reflect the actual number of viral particles (DNA copies) in a blood plasma sample; depending on the test system used.

Presently, the results of studies of genetic polymorphisms of several cytokines are used to determine the sensitivity to treatment of chronic hepatitis C and possibility of spontaneous infection elimination as one of the key factors of the immune system. From this perspective, identification of *polymorphisms of interleukin 28 (IL 28B) gene* is crucial.

Taking into account the current tasks PCR-based analysis is effective in the activities of infection disease specialists, hepatologists, professionals in the area of HIV infection prevention and control.

DNA-Technology offers the following kits (see Table 5) for detection and quantitative analysis of viral hepatitis and HIV as well as HCV typing by PCR.

Table 5. Kits produced by DNA-Technology for detecting viral hepatitis and HIV

Etiologic agent	Detection format				Registration*
	Forez	Flash	Rt	qPCR	
Hepatitis A virus	*	*	–	–	RUO
Hepatitis B virus	*	*	*	*	RU/IVD
Hepatitis C virus	*	*	*	*	RU/IVD
Hepatitis D virus	*	*	–	–	RUO
Hepatitis G virus	*	–	–	–	RUO
Hepatitis C virus genotyping	–	–	*	–	RU/IVD
Human immunodeficiency virus	–	*	*	*	RU/IVD

*** Note:**

RU/IVD – kits for *In Vitro* Diagnostic, which are registered in Russia only

CE/IVD – kits for *In Vitro* Diagnostic, which are registered in EU

RUO – kits for Research Use Only

Kit format:

- Strip tubes (8 pcs., 0.2 ml each);
- Single tubes (0.5 ml or 0.2 ml).

Storage temperature: from +2 to +8 °C.

Shelf life:

- Forez – 9 months (except HBV kit – 12 months);
- FLASH – 9 months (except HBV kit – 12 months);
- Rt – 9 months (except HBV kit – 12 months)

Kits for DNA/RNA extraction:

- *PREP-NA* (for kits used to detect hepatitis B virus DNA, hepatitis C virus RNA, HIV RNA, and for HCV genotyping)
- *PREP-NA* is included in kits for quantitative analysis of hepatitis B virus DNA, hepatitis C virus RNA and Hepatitis C virus genotyping.

Specimen for screening:

- Blood plasma.

Equipment required for analysis:

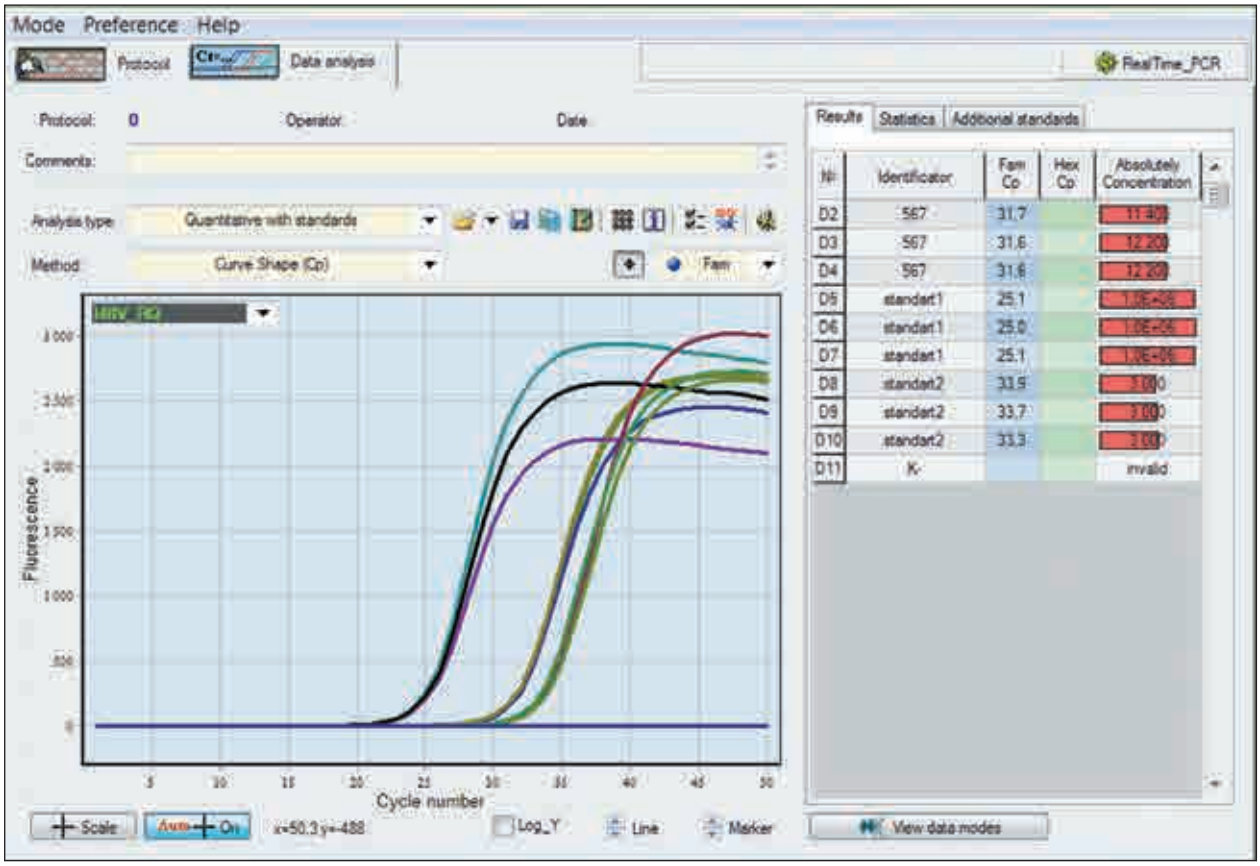
- For *FLASH* kits: Gene, Gene-4 or counterparts
- For *Rt* kits:
  - DT devices produced by DNA-Technology (DTlite, DTprime, DT-96);
  - IQ5 Cycler device produced by Bio-Rad Laboratories and Rotor-Gene Q devices produced by QIAGEN.

The following additional equipment is needed for analysis using strip tubes:

Strip plastic rack and centrifuge (vortex) rotor.

Software: Reaction results are analyzed and interpreted automatically (for devices produced by DNA-Technology) (Fig. 4).

A



B Qualitative analysis

Number of the hole	Identificator of the tube	Cp, Fam	Cp, Hex	Result
D2	567 (HBV_RQ)	31.7		11 400
D3	567 (HBV_RQ)	31.6		12 200
D4	567 (HBV_RQ)	31.6		12 200
D5	standart1 (HBV_RQ)	25.1		1.0E+0.6
D6	standart1 (HBV_RQ)	25.0		1.0E+0.6
D7	standart1 (HBV_RQ)	25.1		1.0E+0.6
D8	standart2 (HBV_RQ)	33.9		3 000
D9	standart2 (HBV_RQ)	33.7		3 000
D10	standart2 (HBV_RQ)	33.3		3 000
D11	K– (HBV_RQ)			invalid

Fig. 4. Results of *Rt* qualitative analysis (DT devices) HBV REAL-TIME PCR Detection Kit
A – Optical measurement analysis (Fam channel)
B – Analysis results

DNA-TECHNOLOGY

5. RESPIRATORY TRACT INFECTIONS

According to WHO, respiratory tract infections are one of the main causes of sickness rate and mortality both at infancy and at older ages. About 70 % of these infections affect the upper respiratory tract and middle ear region.

Diagnosis and treatment of respiratory infections still remain the focus of attention among both therapists and pediatricians since these infections account for over 90 % of all outpatients' complaints, especially during autumn and winter seasons.

Corynebacterium diphtheriae

Corynebacterium is a genus of Gram-positive rod-shaped bacteria that causes diphtheria. Diphtheria is transmitted by air and is characterized by local fibrinous inflammation, especially that of the mucous membranes of the oropharynx and nasopharynx, as well as by general intoxication and visceral injury. Severe consequences of diphtheria are associated with release of toxins into blood and their fixation in tissues, which results in the nervous (parenchymatous neuritis) and cardiovascular (myocarditis) systems disorders.

The difficulties in diagnosing toxigenic strains of corynebacterium diphtheria are associated with the symptoms typical for catarrhal diseases as well as with occurrence of abnormal clinical forms and prolonged asymptomatic carriage of the infectious agent. PCR analysis of diphtheria make it possible to obtain results within 24 hours and determine toxigenicity of the strains of microorganisms, regardless of the beginning of antibiotic therapy.

Specimen for PCR testing:

- Throat swab;
- Tonsillar smear;
- Throat lavage.

Bordetella pertussis

Bordetella pertussis causes whooping cough (pertussis), an acute respiratory disease that is clinically manifested in the form of spasmodic coughing spells, infection of the respiratory, nervous and cardiovascular systems without primary intoxication and temperature reaction. Infected people and bacilli carriers can serve as the infection source. The infection is transmitted by air. It is more common among children under 5, in adults the clinical course is atypical.

The "golden standard" for laboratory diagnosis of pertussis implies inoculation of nasopharyngeal secretions during the first 2-3 weeks of illness; later the test validity declines. Applying serological tests (antibodies to *Bordetella pertussis* antigens and *Bordetella pertussis* toxin) is reasonable at the later stages of infection; the test has high specificity but relatively low sensitivity.

Community-acquired pneumonia is one of the primary causes of people's morbidity, hospitalization and mortality, especially among senior people and those with background diseases.

According to the Russian and foreign researchers, St.pneumoniae is a dominant etiological agent of pneumonia. Among other typical bacterial causative agents of pneumonia a significant etiological role belongs to K.pneumoniae and a group of micro-organisms (obligate and facultative intracellular parasites) resistant to β-lactam antibiotics: mycoplasma pneumonia, chlamidophila pneumonia and legionella pneumophila.

Specimen for PCR testing:

- Epithelial cell scrapings from the nasopharynx;
- Phlegm;
- Aspirate;
- Broncho-alveolar lavage;
- Blood.

Legionella pneumophila

Gram-negative bacterium proliferates in air conditioning systems, showerheads, humidifiers and inhalers and causes infectious disease legionellosis or Legionnaires ' disease. Sporadic cases are rare, as a rule outbreaks occur. Clinical implications are fever, weakness and discomfort, loss of appetite and dry cough. There are two forms of the disease: mild (flu-like) and severe (in the form of pneumonia). Chest X-ray reveals extensive lung damage.

Diagnosis is based on the results of bacteriological inoculation and serological tests (reaction to indirect immunofluorescence). PCR-based diagnostics are used to confirm the diagnosis.

Specimen for PCR testing:

- From patients: blood plasma, mucosal scrapings, broncho-alveolar lavage;
- From the environment

Chlamydophila (Chlamydia) pneumoniae

This is an obligate intracellular parasite. According to the statistics, *C. pneumoniae* is the causative agent of about 5-15 % of the community-acquired pneumonia cases. Prevalence of the disease caused by this infectious agent tends to increase. Mostly *C. pneumoniae* is clinically manifested in acute form – pneumonia, as well as prolonged bronchitis, pharyngitis, laryngitis, sinusitis and arthritis.

Considering the apparent growth of respiratory Chlamydia cases, it is strongly recommended to test at least patients with pneumonia, bronchial asthma and chronic obstructive pulmonary disease (COPD) for *C. pneumoniae*.

Based on the clinical presentation only, it is often impossible to make a clear diagnose. Therefore, special emphasis should be given to laboratory diagnostics to allow for establishing the infection etiology and making diagnostics at early stages of the disease.

Laboratory diagnostics with using microbiological techniques is complicated. The comparison between sensitivity of microscopical method and PCR indicates that the frequency of pathogen detectability by microscopic examination is 10-12 % and by PCR at least 98 % respectively. With meeting the requirements for culture technique, the sensitivity for chlamydia diagnostics is 60-80 %, while PCR shows the sensitivity at least 95-98 %.

Specimen for PCR testing:

- Phlegm;
- Blood;
- Pleural fluid;
- Aspirates from sinus and middle ear;
- Endotracheal aspirates;
- Broncho-alveolar lavage;
- Biopsy specimens.

Mycoplasma pneumoniae

A single-celled Gram-negative microorganism without a cell wall. In terms of structural organization, mycoplasmas lie between bacteria and viruses. The fact that this microorganism can grow in cell-free media and can reproduce themselves excludes them from the virus family. They are regarded as hardly-cultivated microorganisms and considered to be superficial parasites of mucosa cells.

Among the factors that contribute to the development of a pathological process caused by *mycoplasma pneumoniae* there are a patient's age (younger than 5 years old and older than 50), smoking, hypothermia, chronic lung disease, chronic heart disease, chronic kidney disease, chronic gastrointestinal tract disease as well as impairment of overall non-specific human organism resistance (suppression of phagocytosis, bactericidin production, mucociliary clearance disorder, etc.).

The incubation period of the disease can last from 1 to 4 weeks. In most cases, it is only 12-14 days. The disease begins with respiratory syndrome (rhinopharyngitis and/or laryngotracheitis); otitis is possible too. Among extrapulmonary manifestations of mycoplasma pneumonia may be myalgia, sweating, gastrointestinal disorders and CNS disorders (headache and sleep disturbances).

The disease outcome is favorable, however, clinical symptoms and signs regress as well as that of radiographic signs proceeds slowly. At mycoplasma pneumonia a relapse of chronic diseases may occur.

Infection complications are manifested as respiratory distress syndrome, atelectasis, mediastinal adenopathy, pneumothorax, pleural effusion and pulmonary abscess. Extrapulmonary complications manifest themselves in the form of nervous system injury (meningoencephalitis, aseptic meningitis, encephalitis, ascending paralysis and transverse myelitis); haematological diseases (autoimmune hemolytic anemia, paroxysmal cold hemoglobinuria, Raynaud's phenomenon, disseminated intravascular coagulation, thrombocytopenia), cardiac complications (pericarditis, myocarditis, heart failure, complete AV block), skin and mucous membrane lesions (maculopapular rash and vesicular lesions; patients with rash may develop aphthae and conjunctivitis); articular manifestations (arthritis).

Differential diagnostics is performed in case of various pneumonias: viral, bacterial, fungal, pneumocystis, and also at tuberculosis.

Clinical, radiological and laboratory data regarding M.pneumoniae-caused infections are inadequate in order to make an accurate diagnosis based on them. Therefore laboratory diagnostics should include direct methods (first and foremost PCR) for detecting the microorganism in the biological material.

Specimen for PCR testing:

- Phlegm;
- Blood;
- Pleural fluid;
- Aspirates from sinus and middle ear;
- Endotracheal aspirates;
- Broncho-alveolar lavage;
- Biopsy specimens.

Streptococcus pneumoniae

Streptococcus pneumoniae is a natural inhabitant of a human upper respiratory tract. Streptococci are Gram-positive facultative anaerobic microorganisms. Pneumococci are characterized by the presence of strong polysaccharide capsule, which act as protection, inhibiting opsonization and subsequent phagocytosis. There are at least 90 different capsular types of *S. pneumoniae*, but the majority (>90 %) of invasive diseases are caused by 23 serotypes that are included as compounds in currently used polysaccharide vaccine.

β-hemolytic streptococci (that rarely causes pneumonia with healthy adults, as a rule against the background of diabetes or other serious illnesses) and pyogenic streptococci (that often causes pneumonia among children and young people) penetrate into the lungs airborne.

Streptococcus pneumoniae is rarely seen in adults and considerably more often (20 % of cases) in children. In the focal pneumonia pattern with healthy (non-immunosuppressed) people the share of the infection can amount to 10 %. In general this microbe causes mostly otitis, tonsillitis and pharyngitis. In rarer cases, pneumococcus can cause other localized infections (endocarditis, septic arthritis, primary peritonitis, cellulitis, etc.).

Bacterial inoculation is recognized as the golden standard for detecting the microorganism. PCR is used to confirm diagnosis and detect the microorganism against the background of commenced antibiotic therapy.

Specimen for PCR testing:

- Phlegm;
- Blood;
- Pleural fluid;
- Aspirates from sinus and middle ear;
- Endotracheal aspirates;
- Broncho-alveolar lavage;
- Biopsy specimens.

DNA-Technology offers the following kits (see Table 6) for detecting bacterial respiratory tract infections by PCR.

Table 6. Kits produced by DNA-Technology for detecting bacterial respiratory tract infections

Etiologic agent	Detection format				Registration*
	Forez	Flash	Rt	qPCR	
Chlamydia pneumoniae	*	–	*	–	RUO
Bordetella pertussis	*	*	*	–	RUO
Corynebacterium diphtheriae	*	*	*	–	RUO
Legionella pneumophila	*	*	*	–	CE/IVD
Mycoplasma pneumoniae	–	–	*	–	RUO
Streptococcus pneumoniae	–	–	*	–	RUO
Chlamydia pneumoniae/ Mycoplasma pneumoniae	–	–	*	–	RUO

*** Note:**

RU/IVD – kits for *In Vitro* Diagnostic, which are registered in Russia only

CE/IVD – kits for *In Vitro* Diagnostic, which are registered in EU

RUO – kits for Research Use Only

Kit format:
- Strip tubes (8 pcs., 0.2 ml each);
- Single tubes (0.5 ml or 0.2 ml).

Storage temperature: from +2 to +8 °C.

Shelf life:
- Forez – 9 months;
- FLASH – 12 months;
- Rt – 12 months.

DNA extraction kits:
- *PREP-RAPID;*
- *PREP-NA (for RNA extraction);*
- *PREP-GS.*

Specimen for screening:
- Vesicles fluid;
- Scrapings from mucosa;
- Phlegm;
- Cerebrospinal fluid;
- Bronchial aspirate;
- Biopsy specimen;
- Blood;
- Surface swabs.

Recommended additional reagents:
SIC-sample intake control- is designed for DNA identification (qualitative analysis) and approximate estimation of the amount of human genomic DNA (quantitative analysis) in a human biomaterial by real-time PCR.

Equipment required for analysis:
- For *FLASH* kits: Gene, Gene-4 or counterparts
- For *Rt* kits:
  - DT devices produced by DNA-Technology (DTlite, DTprime, DT-96);
  - IQ5 Cycler device produced by Bio-Rad Laboratories and Rotor-Gene Q devices produced by QIAGEN.

The following additional equipment is needed for analysis using strip tubes:
Strip plastic rack and centrifuge (vortex) rotor.

Software: Reaction results are analyzed and interpreted automatically (for devices produced by DNA-Technology).

Influenza (flu) *is an acute infectious disease of the respiratory tract caused by the influenza virus. It is an acute respiratory viral infection* (**ARVI**). In terms of frequency and number of flu incidences, ARVI ranks first in the world, accounting for 95 % of all infectious diseases. The death rate from this disease remains high: 2 million people in the world die each year from flu. In Russia, per every 100 thousand people there are 2.7 children's deaths from influenza and 80 cases of deaths among persons older than 65.

Influenza viruses belong to the *Ortomyxoviridae* family, which includes 3 genera: *influenza A virus, influenza B virus* and *influenza C virus*. The antigenic properties of internal virion proteins identify influenza virus as genera A, B or C. Further grouping is done according to subtypes of surface proteins hemagglutinin and neuraminidase.

Viruses containing three hemagglutinin subtypes (H1, H2, H3) and two neuraminidase subtypes (N1, N2) are of epidemic concern for people. Influenza viruses A and B contain hemagglutinin and neuraminidase as the main structural and antigenic components of viral particles that have hemagglutinating and neuraminidase activities. Influenza C virus has no neuraminidase. It has a hemagglutinin-esterase (penetrating) protein (HEF).

Influenza A virus

It is worldwide and causes seasonal epidemics among humans, outbreaks of animal disease (H1N1 swine flu) and bird disease (H5N1 (bird flu). It regularly causes pandemics. The features of influenza type A virus genome are associated with a high degree of variability (point mutations, antigenic shift) that causes a variety of subtypes.

Influenza A viruses (H1N1 and H3N2 subtypes) that cause seasonal epidemics usually circulate among humans. There are also cases of human infection with influenza A viruses of other subtypes that are more characteristic for birds. Some of these subtypes (for example, H5N1) have pandemic potential.

Influenza B virus

When infected with influenza B virus the disease usually proceeds in a milder form, affecting mostly children and young adults. A characteristic feature of influenza B viruses is that it circulates only in human environment.

DNA-Technology offers the following kits (see Table 7) for detecting viral respiratory tract infections by PCR.

Table 7. Kits produced by DNA-Technology for detecting viral respiratory tract infections

Etiologic agent	Detection format				Registration*
	Forez	Flash	Rt	qPCR	
Influenza A virus (subtype H5N1)	–	*	*	–	RUO
Influenza A virus (subtype H1N1)	–	–	*	–	CE/IVD
Influenza A virus	–	–	*	–	CE/IVD
Influenza B virus	–	–	*	–	CE/IVD
Influenza A&B virus	–	–	*	–	CE/IVD
Influenza A virus, Influenza B virus Multiplex	–	–	*	–	RU/IVD
Acute viral respiratory infections Multiplex	–	–	*	–	RU/IVD

*** Note:**

RU/IVD – kits for *In Vitro* Diagnostic, which are registered in Russia only

CE/IVD – kits for *In Vitro* Diagnostic, which are registered in EU

RUO – kits for Research Use Only

Kit format:
- Strip tubes (8 pcs., 0.2 ml each);
- Single tubes (0.5 ml or 0.2 ml).

Storage temperature: from +2 to +8 °C (Taq-polymerase and reverse transcriptase at –20 °C).

Shelf life:
- FLASH – 12 months;
- Rt – 9 months (except influenza A virus kit (subtype H5N1) – 12 months)

Kits for RNA extraction:
- *PREP-NA;*
- *PREP-GS* (for detecting influenza B virus)

Specimen for PCR study:
- Human biomaterial (smears and swabs from the nasal cavity and oropharyngeal cavity);
- Material from sick and dead animals (smears and swabs from the trachea, nasal cavity, throat, cloacae; feces, internal organs).

Equipment required for analysis:
- For *FLASH* kits: Gene, Gene-4 or counterparts
- For *Rt* kits:
  - DT devices produced by DNA-Technology (DTlite, DTprime, DT-96);
  - IQ5 Cycler device produced by Bio-Rad Laboratories – only for influenza A virus (H5N1 subtype (bird flu)).

The following additional equipment is needed for analysis using strip tubes:
Strip plastic rack and centrifuge (vortex) rotor.

Software: Reaction results are analyzed and interpreted automatically (for DT devices produced by DNA-Technology) (fig.5).

A

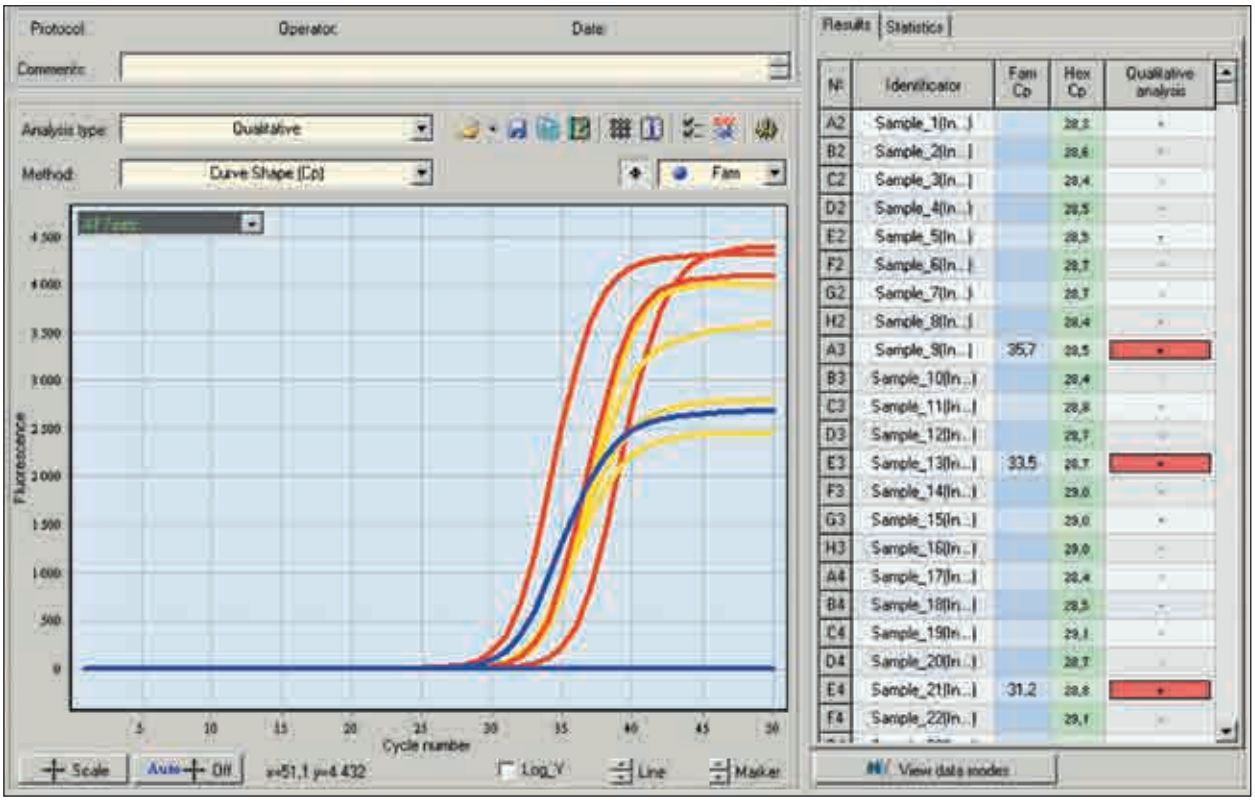


Fig. 5. Analysis results for Rt optical measurements (DT devices)
Influenza B virus Real time PCR Kit
A – Optical measurement analysis (Fam channel)

6. ESPECIALLY DANGEROUS AND NATURAL FOCAL INFECTIONS

Especially dangerous infections (EDIs). According to the International Health Regulations (IHR), EDIs are "infectious diseases that are included in the list of events which may constitute a public health emergency of international concern".

Annex 2 of IHR-2005 defines a list of such infections, divided into two groups. The first consists of "diseases, which are unusual or unexpected and may have serious public health impact": smallpox, poliomyelitis due to wild-type poliovirus, human influenza caused by a new subtype and severe acute respiratory syndrome (SARS).

The second group is defined as "diseases that have demonstrated the ability to cause serious public health impact and to spread rapidly internationally": cholera, pneumonic plague, yellow fever, viral hemorrhagic fevers and West Nile fever.

Natural feral herd infections are a special group of diseases that have evolutional lesions. Natural focus implies a biotope on the territory of a particular geographical landscape inhabited by animals whose species or interspecies differences support the pathogen circulation due to its transmission from one animal to another through blood-sucking arthropod vectors.

At the moment, an adverse epidemic situation for infections that were previously endemic in nature is forming. That is why, in order to take antiepidemic measures timely it is vital to apply quick-tests that can have the following characteristics:

- Obtaining the test results in the shortest possible time;
- Ability to carry out and complete analysis without isolating the required microorganism in a pure culture using native materials only;
- High specificity and sensitivity as a prerequisite for appropriate reliability of analysis;
- High efficiency, simplicity, availability and reproducibility of the analyses.

Borrelia burgdorferi

This is a microbial species belonging to the Spirochaetaceae family. The species contains 36 different types. 12 of these Borrelia species cause various diseases in humans – borrelioses (Lyme disease, relapsing fever) mainly of zooantroponoze nature with transmissible way of transferring the pathogen.

In Lyme disease, the major etiologic factors are *Borrelia burgdorferi*, *Borrelia afzelii*, *Borrelia garinii* and *Borrelia valaisiana* species. Relapsing fever is often accompanied by bacteremia. Its pathogen – Borrelia recurrentis, which enters a human body through a bite of an insect infected.

The main transmission vectors are:

- Ticks – ixodic ticks (*Ixodes scapularis*), argasidae ticks (*Ornithodoros papillares* (village tick) and *Argas persicus* (Persian tick));
- Head lice, body lice and pubic lice.

Complex inflammatory and allergic skin changes, manifested in the form of specific erythema that is characteristic of Lyme disease usually develop at the site of tick bites.

The disease can begin with ill health, itching, swelling and redness at the site of a tick bite. As the disease progresses (or immediately in patients without local phase), borrelia spreads through the blood and lymph from the bite site to the internal organs. The infection symptoms develop within 1-1.5 months.

Borrelia can remain in a human body for a long time, causing chronic and relapsing disease. Lyme disease diagnosis at the late stage is especially difficult because of the severity of clinical polymorphism.

Laboratory diagnostic methods are essential in establishing the diagnosis of latent, subclinical forms and at the later stages. With PCR analysis, early diagnosis is possible during the serological window period.

Specimen for PCR testing:

- Infected tissues and body fluids of the patient;
- Ticks.

Vibrio cholerae

Vibrio cholerae is a Gram-negative, facultative anaerobic motile bacterium of the genus *Vibrio*. There are more than 140 serogroups of *V. cholera* at present; they are divided into agglutinated and non-agglutinated with generic cholera serum O1 (*V. cholerae non O1*). "Classical" cholera is caused by Vibrio cholerae serogroup O1, which includes two biovars: classic (*Vibrio cholerae biovar cholerae*) and El Tor (*Vibrio cholerae biovar eltor*).

Cholera is an acute infectious disease related to highly dangerous infections. It is characterized by fecal-oral mechanism of infection, small intestinal lesion, watery diarrhea, vomiting, rapid loss of body fluids and electrolytes with development of various degrees of dehydration up to hypovolemic shock and death.

The incubation period lasts from several hours to 5 days, usually 24-48 hours. The severity of the disease varies from latent and subclinical forms to severe conditions with sharp dehydration and death within 24-48

hours. In this regard, very fast and highly sensitive method, such as PCR analysis, is required for diagnostics.

Specimen for PCR testing:

- Feces and/or vomit, water.

Bacillus anthracis

Bacillus anthracis is a Gram-positive, spore-forming bacterium. It is an etiologic agent of anthrax. *Bacillus anthracis* is especially dangerous infectious disease of farm and wild animals of all species, and, occasionally, of humans. The disease occurs at lightning speed, hyperacute, acute and subacute – in sheep and cattle; acute, subacute and anginal – in pigs; predominantly as anthrax – in humans. It is characterized by intoxication, serous-hemorrhagic inflammation of skin, lymph nodes and internal organs; it occurs in a skin or septic form (intestinal and pulmonary forms are found in animals).

Diagnosis is based on clinical, epidemiological and laboratory data. Laboratory diagnosis includes bacterioscopic and bacteriological methods. But for the purpose of early diagnosis, immunofluorescence and PCR methods are used for laboratory diagnosis.

Specimen for PCR testing:

- Fluid of vesicles and carbuncles;
- Phlegm;
- Blood;
- Feces;
- Vomit (septic form).

Yersinia pestis

Yersinia pestis is a Gram-negative bacterium from the Enterobacteriaceae family. It is the infectious agent of bubonic plague and can also cause pneumonia (pulmonary form of plague) and septic plague. All the three forms are responsible for high mortality rate during epidemics.

Plague is an acute natural focal infectious disease in the group of quarantine infections. It occurs with very severe general condition, fever, damage to the lymph node, lung and other internal organs, often with development of sepsis. The disease is characterized by high mortality rates and extremely high infectivity.

In natural foci, the sources and reservoirs of the infectious agent are rodents – groundhogs, gophers and sandworts, mouse-like rodents and rats (gray and black). The rarer ones are house mice, and also double-toothed rodents, cats and camels. Vectors of the infectious agent are flea of different types.

Specimen for PCR testing:

- Lymph node festering punctate;
- Phlegm and blood of the patient;
- Discharge from fistulas and ulcers.

Francisella tularensis

Francisella tularensis is a Gram-negative bacillus with polymorphism. It is a causative agent of tularemia – a zoonotic disease that has natural foci. It is characterized by intoxication, fever and lymph node damage. It occurs in 3 forms, namely, bubonic, pulmonary and generalized (spreads throughout the body) forms. Tularemia bacillus vectors are hares, rabbits, water rats and voles. Epizootic outbreak periodically occurs in the natural foci. The infection is transmitted to humans through contact or directly from animals, or through contaminated food and water. Seldom infecting occurs through aspiration (when threshing bread and handling grains and forage products) and via blood-sucking arthropods (horsefly, ticks, mosquitoes, and others).

Bacteriological diagnostic methods for human tularemia have additional significance and are not always effective due to the biological characteristics of the pathogen and characteristics of human infection (low concentration of the infectious agent in tissues and organs). A much more efficient diagnosis method is the PCR method, which can significantly reduce the time of analysis.

Specimen for PCR testing:

- Bubo punctate, conjunctival discharge, film with tonsil, phlegm and others (from humans);
- Biopsy specimen (from animals).

DNA-Technology offers the following kits (see Table 8) for detecting especially dangerous and natural focal infections by PCR.

Table 8. Kits produced by DNA-technology for detecting especially dangerous and natural focal infections

Etiologic agent	Detection format				Registration*
	Forez	Flash	Rt	qPCR	
Borrelia burgdorferi	–	*	*	–	RU/IVD
Vibrio cholerae	–	*	*	–	RUO
Bacillus anthracis	–	–	*	–	RUO
Yersinia pestis	–	*	*	–	RUO
Francisella tularensis	–	*	–	–	RUO

*** Note:**

RU/IVD – kits for *In Vitro* Diagnostic, which are registered in Russia only

CE/IVD – kits for *In Vitro* Diagnostic, which are registered in EU

RUO – kits for Research Use Only

Kit format:

- Strip tubes (8 pcs., 0.2 ml each);
- Single tubes (0.5 ml or 0.2 ml).

Storage temperature: +2 to +8 °C.

Shelf life:

- Forez – 9 months;
- FLASH – 12 months;
- Rt – 12 months.

DNA extraction kits:

- *PREP-RAPID;*
- *PREP-NA;*
- *PREP-GS.*

Specimen for screening:

- Scrapings from mucosa;
- Faeces;
- Cerebrospinal fluid;
- Biopsy specimen;
- Blood;
- Vomit;
- Phlegm;
- Vesicles fluid.

Equipment required for analysis:

- For *FLASH* kits: Gene, Gene-4 or counterparts
- For *Rt* kits:
  - DT devices produced by DNA-Technology (DTlite, DTprime, DT-96);
  - IQ5 Cycler device produced by Bio-Rad Laboratories.

The following additional equipment is needed for analysis using strip tubes:

Strip plastic rack and centrifuge (vortex) rotor.

Software: Reaction results are analyzed and interpreted automatically (for devices produced by DNA-Technology) (fig.6).

A

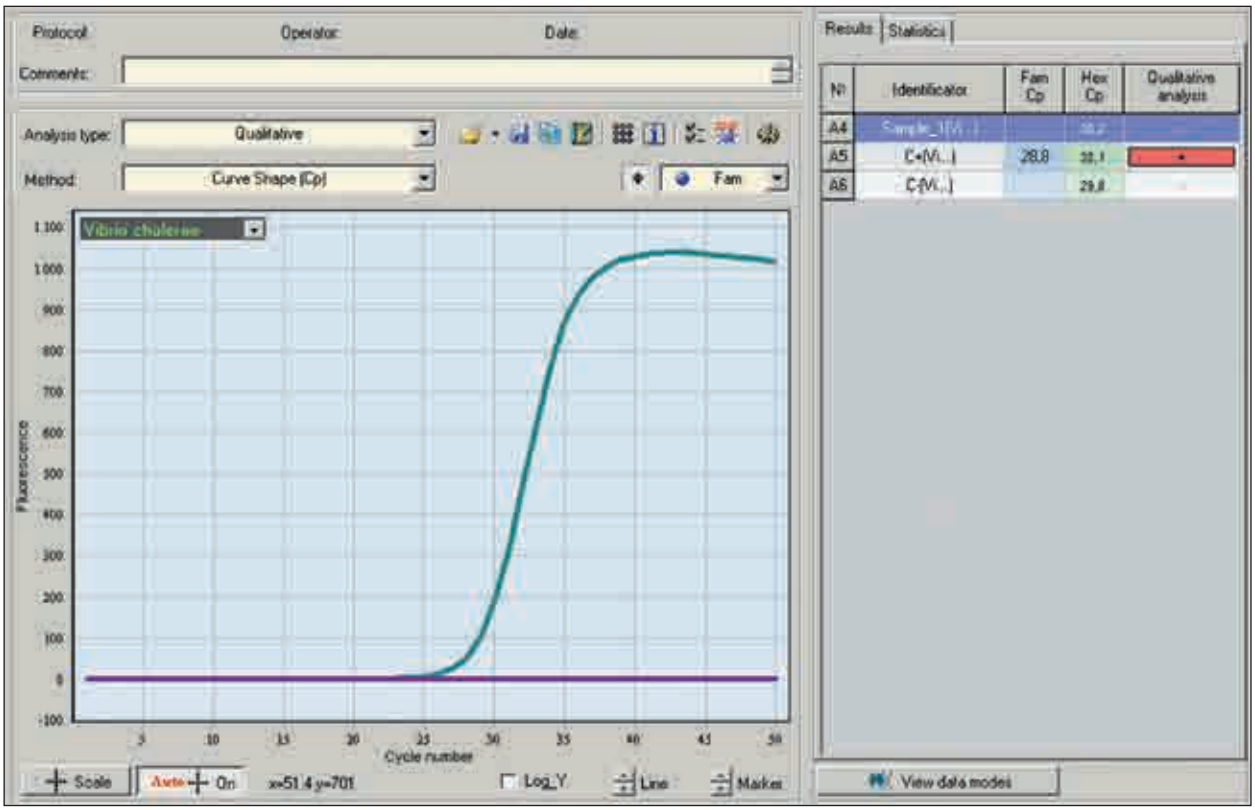


Fig. 6. Analysis results for Rt optical measurements (DT devices)
Vibrio cholera PCR Detection Kit
A – Optical measurement analysis (Fam channel)

7. OTHER INFECTIONS

MRS/MRSA Multiplex PCR Detection kit

The kit for detection of the DNA of *Staphylococcus spp.*, *Staphylococcus aureus* and *mecA* gene, which causes methicillin-resistance, by PCR in Real-time (MRS/MRSA).

In recent decades, the strains of pathogens that are resistant to the action of various drugs have become widespread worldwide. The resistance of microorganisms to the antibiotics can be natural or acquired, but forming the resistance in both cases is based on genetic factors.

Infectious disease caused by strains, which are resistant to antibiotics, are characterized characterized by longer duration, often require hospitalization with long stay in the hospital.

There are widely spread and well known methicillin-resistant strains of *Staphylococcus spp.* (**MRS** – methicillin-resistant *Staphylococcus*), including *Staphylococcus aureus* (**MRSA** – methicillin-resistant *Staphylococcus aureus*), coagulase-negative *Staphylococcuses: S. epidermidis, S. saprophyticus* and others (**MRCoNS** – methicillin-resistant coagulase-negative staphylococci).

Resistance to methicillin and oxacillin is caused by presence of penicillin binding protein (*PBP2A – penicillin binding protein 2A*) in the microorganism, which is coded by the *mecA* gene, transmitted as part of the mobile element ssc (*staphylococcal cassette chromosome*).

S. aureus – Gram-positive Staphylococcus spp, has a golden color due to the carotenoid pigments. In approximately 25-50 % of people asymptomatic colonization of S.aureus is present on the skin and mucous. In 30-60 % of cases, it leads to the development of infection in the presence of certain factors and conditions. These are:

- reception of various medications (immuno-suppressants, hormones, antibiotics, etc.);
- the impact of adverse environmental factors;
- decreased immunity;
- hospital treatment;
- pregnancy and childbirth;
- old age and the child age;
- traumatic condition (mechanical damage to the integrity of the skin, burns, etc.);
- chronic diseases (diabetes, cancer, etc.).

S. aureus remains one of the most important pathogens of human infections, causing a wide range of community-acquired and nosocomial diseases, from skin and soft tissue lesions to pneumonia, endocarditis, sepsis and toxic shock syndrome.

Path of transmission:

- airborne;
- airborne dust;
- contact;
- food;
- an artificial (related to medical procedures).

The ubiquity of S. aureus, the active colonization of the human, affected individuals of all age groups, etiological significance for a variety of diseases, especially heavy – all this determines the importance of a correct choice of drugs for the treatment. β-lactam antibiotics (oxacillin and methicillin) are traditionally included in the starting antibiotic therapy. It is therefore important to quickly get to the appointment of treatment and be sure to take into account the sensitivity spectrum of S. aureus.

According to the results of epidemiological monitoring of MRSA, it was found that the prevalence of methicillin-resistant strains has been uneven and varies from 2.6 % to 60 % of all isolates of S. aureus strains. The risk of death increases nearly three-fold times among patients whose bacteremia is caused by MRSA, compared with patients infected with methicillin-sensitive strains of S. aureus [8].

Other methicillin-resistant *Staphylococcuses* (for example MRCoNS, and especially *S. epidermidis*) – are microorganisms often causing lesions of shunts, joint prostheses, urinary and venous catheters and other serious nosocomial infections such as sepsis and opportunistic disease in patients with reduced immunity.