DNA-TECHNOLOGY

CATALOGUE EQUIPMENT

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Moscow

INTRODUCTION

Dear Colleagues and Friends!

Now you are holding a jubilee catalogue dedicated to the results of work of our company during 20 years. Within this period we have developed and introduced into medical practice the following unique projects:

1993 – TercycMulti-Channel PCR Thermal Cycler

The unique technical specifications of the device are unequalled in their class. The device is characterized with high reliability, compact design, flexible control and an affordable price, which provides for its growing popularity both in Russia and CIS countries. The development and introduction into laboratory practice of Tercyc made PCR-analysis more affordable for many research and clinical diagnostic laboratories. By now more than 3 800 devices are in operation in Russia and CIS countries.

2001 – FLASH Technology.

A unique technology, which, at the moment of its development, was one-of-a-kind in the world. For detection the technology implements the signal of fluorescent marked probes at the end of thermal cycling process. The technology includes both reagents and the unique Fluorescence Detector "Gene", which combination makes it possible to solve an important problem of clinical laboratories, namely, reducing the contamination risk. As a result, the work of laboratory scientists is simplified and the requirements to PCR-laboratories are leveled down.

FLASH Technology is a powerful stimulus for opening new PCR-laboratories and increasing the number of PCR-analyses in clinical practice.

2005 – DT-322 Real-Time PCR Thermal Cycler

DT-322 is the first Russian Real-Time PCR Thermal Cycler. This device provided affordability for clinical laboratories of quantitative PCR-analysis, which is required during diagnosis and control of such socially significant diseases as AIDS, hepatitis B and C.

2007 – DTprime Real-Time PCR Thermal Cycler

DTprime is the first Russian Real-Time PCR Thermal Cycler with four channels and 96 wells. DT–96 became the 'parent' of a series of devices, which led Russian manufacturing of medical equipment in the field of PCR onto the level of world standards. With the development of DT-96 there arose the opportunity for creating fully automatic PCR-blocks with implementation of Russian devices.

By now more than 650 thermal cyclers of DT series have been installed and are in operation in clinical and research laboratories.

2008–2009 – Femoflor® Real-Time PCR Kit.

The company developed and patented the first in the world reagents kit for analyzing biocenosis of the female urogenital tract by the PCR-method.

Clinical doctors acquired an analytically accurate and technological tool for qualitative and quantitative analysis of complex microbial communities. By the present time more than 50 000 pa-

tients have been examined, with the reagent kit Femoflor® being the primary tool for diagnostics of dysbioses of various etiology. The Ministry of Health of the Russian Federation provides this reagent kit within the program of high-tech assistance in the field of Obstetrics & Gynaecology.

2010–2011 – Real-Time PCR Kits for detecting of genetic polymorphisms associated with the risk of development of multifactorial disorders.

At present, the most popular direction of PCR-implementation is analyzing the human genetics, namely, predispositions to various multifactorial disorders. At present, the human genome is believed to have up to 10 million SNPs (Single Nucleotide Polymorphisms, which are slight changes in the genome involving 1–2 neighboring polymorphisms). Single nucleotide polymorphisms, as well as larger genetic damages (deletions, chromosomal aberrations), are a risk factor of development of many disorders and provide for the efficiency of metabolism of various substances.

2012–2013 – of The market introduction of automatic PCR station wich include DT–384 Real-Time PCR Thermal Cycler, liquid handling device, plate sealer and operating software.

Up-to-date development of laboratories requires improving the accuracy and increasing the speed of analysis. In connection with these requirements there have been developed automatic systems adapted for 384-well microplates

We are grateful to you that throughout these years you have stayed close by, supported us in our undertakings, applied to us with questions and suggestions, taught us and been taught by us, improved PCR-technology, invited us to conferences and taken part in our events. We hope that the period of the next 20 years will be even more productive and eventful.

General Director D. Yu. Trofimov
Personnel of "DNA-Technology" Multicorporate Enterprise

ABOUT THE COMPANY

"DNA-Technology" Multicorporate Enterpise has been developing, manufacturing and integrating high-tech equipment and reagents for PCR applications since 1993.

The company's personnel includes leading specialists in the field of molecular biology, immunogenetics, medicine, thermodynamics, optics, electronics, programming, which provides for a high scientific and technological potential of the company and high standards of quality and production control at all its stages.

"DNA-Technology" production base meets all modern requirements made to companies which operate in the field of production of medical equipment. This is testified by the license issued by the Federal Service for Supervision in Health Care and Social Development; certificate testifying that the quality management system as regards the production of medical devices and reagents for laboratory diagnostics correspond to the requirements of GOST ISO 9001 – 2001 (9001:2000), and quality management certificates (ISO 13485:2003 и 9001:2008).

The main directions of "DNA-Technology" activities are the following:

- creating common technological solutions for PCR-laboratories – from the laboratory plan, supplies of equipment and reagents to promotion of a wide range of research, training of laboratory personnel, interpretation of the results obtained and work with clinicians;
- developing and producing high-tech equipment for conducting diagnostic and scientific analysis by PCR-method;
- producing a wide range of reagents for needs of clinical bacteriology, virusology and gene diagnostics;
- producing a wide range of reagents for detecting DNA of infectious agents of agricultural plants and diagnostics of genetically modified organisms (GMO);
- providing service support;
- cooperation with clinicians and scientists.

The range of products includes main equipment for PCR-laboratories:

- Real-Time PCR Thermal Cyclers (DT-series devices);
- Tercyc PCR Termal Cycler for end-point detection (FLASH and electrophoretic detection);
- fluorescence detectors for FLASH PCR Kits ("Gene" series devices);
- thermostats;
- power supplies ("Elf" series devices);
- PCR-boxes.

The company created a strong R&D basis used for developing highly sensitive and specific PCR kits:

- detecting viral and bacterial infections:
  - hepatitis and HIV;
  - urogenital infections;
  - herpes viral infections;
  - papilloma viral infections;
  - respiratory tract infections;
  - especially dangerous and natural focal infections;
  - other infections;
- analyzing the disbiotic conditions of the urogenital tract;
- detecting GMO;
- detecting the genotype of humans and microorganisms.

"DNA-Technology" develops unique and one-off for the Russian market technologies of gene diagnostics and predisposition to a number of disorders which lead to polyorganic pathologies, oncologic diseases, disorders of metabolic processes, malfunctioning of the immune system, and detecting inherited disorders.

Specialists' high qualification and equipment of the laboratory basis make it possible for "DNA-Technology" to train specialists in laboratory diagnostics in the field of modern molecular genetic methods and to consult them on issues of PCR- analysis and further interpretation of the results.

TERMINOLOGY

Allele (*from Greek 'allelon' – one another*) is alternative forms of a gene at corresponding sites (loci) on homologous chromosomes, which determine alternative inherited traits. All genes of somatic cells, excluding genes on sex chromosomes, have two alleles, one of which is inherited from the male parent and the other – from the female parent.

Gene (*from Greek 'genos' – generation, race*) is a hereditary unit consisting of a sequence of DNA that determines the structure and synthesis features of a protein molecule.

Genetic polymorphism is the occurrence of two or more different hereditary forms within one population, a gene section with more than one variant of nucleotide sequence existing. The most widely spread is single nucleotide polymorphism, which is replacement of one nucleotide with another one at a certain site of the genome.

Genotype (*from Greek 'genos' – generation, race + 'typos' – dent, impression, mark*) is the entire combination of alleles of a gene or a group of genes responsible for the analyzed trait of an individual.

Heterozygous genotype (*heterozygous state of a gene*) is a genotype having different alleles of the same gene.

Hyperergy (*from Greek 'hyper' – over, beyond, and 'ergon' – action*) is increased functional activity, hypersensivity.

Homozygous genotype (*homozygous state of a gene*) is a genotype having the same alleles of the same gene.

Multifactorial disorders (*disorders with genetic predisposition*) are disorders which develop as a result of interaction between certain genetic factors and specific impacts of environmental factors.

Nucleotide is a complex chemical compound in its natural state. It consists of a nitrogenous base, a sugar (ribose or deoxyribose), and a phosphate group. A single component of molecules of nucleic acids (DNA and RNA). There are four types of nucleotides, the combination of which forms a nucleotide sequence: **A** (*adenine*), **G** (*guanine*), **T** (*thymine*), **C** (*cytosine*), which is a DNA sequence; **A** (*adenine*), **G** (*guanine*), **T** (*thymine*), **U** (*uracil*), not present in DNA, a replacement of thymine in RNA), which is a RNA sequence.

Positive predictive value is the probability that a positive test reflects the underlying condition being tested for.

Risk factors are the general term for factors which are not the direct cause of a certain disorder, but which increase the probability of its occurrence. They can be modified (behavioral) and non-modified (physiological).

Phenotype (*from Greek 'phainon' – revealing and 'typos' – mark*) is a whole combination of genotype expressions (observable characteristics of an individual), and in its narrow sense, certain traits (phenes), controlled by certain genes. The term 'phenotype' encompasses any traits of an organism, starting from primary products of acting genes, RNA molecules and polypeptides, and ending with special features of appearance, physiological processes, behavior, etc. The phenotype is formed on the basis of interaction between the genotype and a number of environmental factors.

OR is odds ratio. It is determined as the ratio of odds of occurrence of a result under the impact of a given risk factor to the odds of occurrence of the result with no impact of the risk factor. Here, it is used for estimation of the odds of occurrence of a certain clinical condition depending on the genotype of an individual. OR>1 corresponds to an increased risk of occurrence of the result being analyzed, while OR<1 – to a decreased risk.

CONTENT

METHOD

I. METHOD: PCR IN CLINICAL LABORATORY DIAGNOSTICS

Polymerase chain reaction (PCR) is the most effective method of molecular diagnostics, which is widely spread in the field of in vitro laboratory analysis. In the EU countries the share of PCR amounts up to 80 % of laboratory analysis, while more than 40 mln analyses using this technology are held in Russia per year.

1. PRINCIPLE OF PCR

PCR is an experimental method of molecular biology which makes it possible to achieve a significant increase of even small concentrations of certain fragments of nucleotide acid (DNA/RNA), acquired during collecting and isolating DNA from the biological material (sample).

PCR operational principle is based on *in vitro amplification* of the given fragments of DNA with a completely or partially known sequence.

Realization of this principle requires, first of all, presence of a reaction mixture the content of which should include the following components:

- *Primers*, which are artificially synthesized oligonucleotides, that, as a rule, have the size from 15 to 30 nucleotides identical to the corresponding sections of DNA-target. They play the key role in formation of amplification reaction products.
- *Taq-polymerase*, which is a thermostable enzyme providing for completing 3'-end of DNA second chain in accordance with the complementarity principle.
- *A combination of deoxynucleotidetriphosphates (dNTPs)*:
  - deoxyadenosine triphosphates (dATPs);
  - deoxyguanosine triphosphates (dGTPs);
  - deoxycytosine triphosphates (dCTPs);
  - deoxythymidine triphosphates (dTTPs).

It is the "construction material" used by Taq-polymerase for DNA chain synthesis.

- *Buffer*, which is a combination of cations and anions in a certain concentration providing for optimal conditions for the reaction as well as stable pH-value.
- *Template*, which is prepared for adding to the reaction mixture sample that can contain the target DNA, e.g. DNA of microorganisms being a target for further multiple copying. With no DNA target the specific product of amplification is not formed.

The amplification process consists of three steps:

1. Denaturation (it is a transformation of double-stranded DNA into single-stranded during disruption of hydrogen bonds between complementary couples of bases under the impact of high temperatures (Fig. 1).

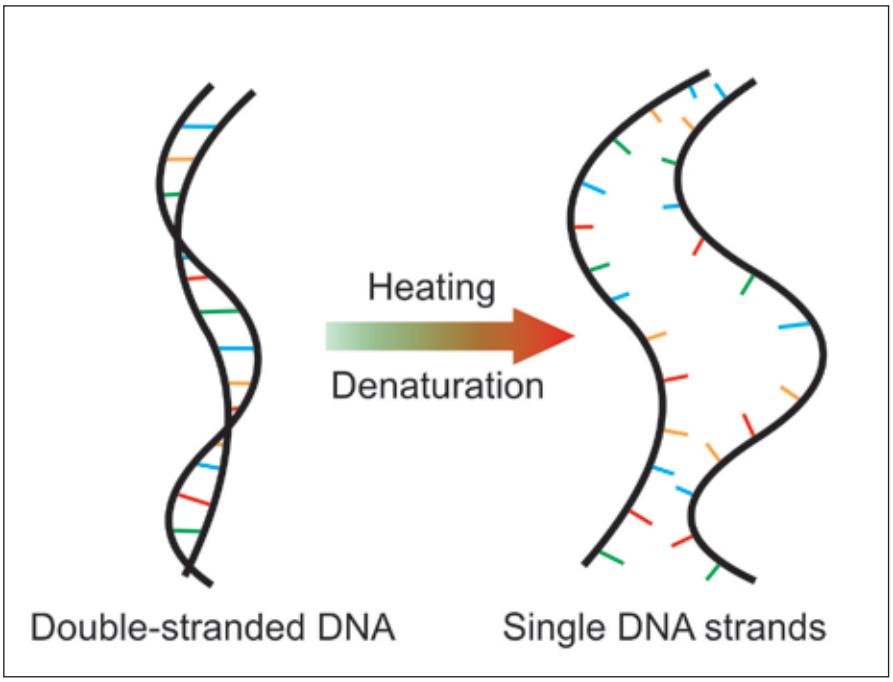

Fig. 1. DNA Denaturation

2. **Annealing** (it is connection of primers to the single-stranded DNA-target. Primers are selected by reagent producers in such a way so that they flank the target fragment and complementary to specific DNA sequence.

3. **Elongation** (extension). Following the annealing of primers Taq-polymerase starts to complete the DNA second strand starting from 3′- end of the primer.

The reaction temperature is increased to the optimum one for Taq-polymerase, which starts synthesis of DNA second strand starting from 3′- end of the primer connected with the matrix and moves on in the direction from 3′ to 5′ end (Fig. 2).

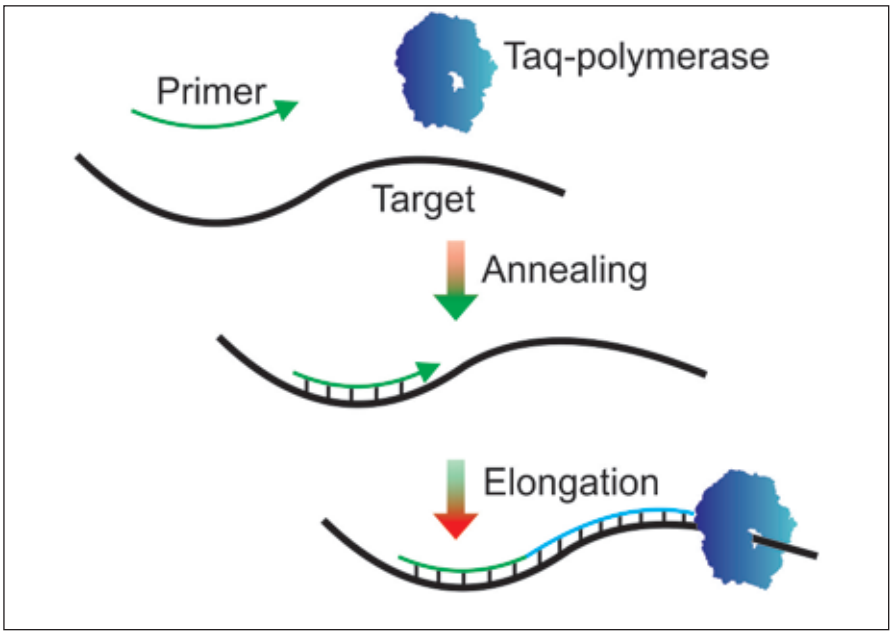

Fig. 2. Primer Annealing and Elongation

The amplification temperature cycle repeats itself several times – 30 times or more thus providing the sufficient amount of specific PCR product (Fig. 3).

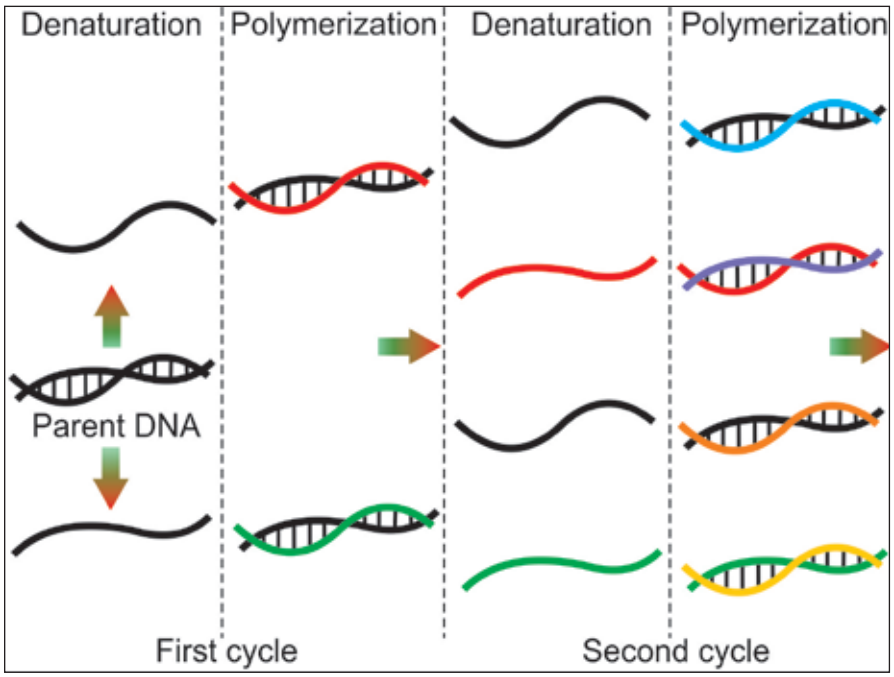

Fig. 3. PCR Steps

During each cycle the number of synthesized copies of DNA fragments doubles. The process of accumulation of specific products of amplification goes exponentially, and then its efficiency falls critically – *plateau effect*.

For the purpose of convenience of detection or control of the amplification process efficiency the reaction mixture can include additional components:

- *DNA-probes*, which are artificially synthesized oligonucleotides of a small size (up to 30 nucleotides) complementary to specific amplicons (PCR products). Presence of isotopic or fluorescent tags in the probe makes it possible to detect reaction products.

During internal laboratory control over the quality of laboratory analysis one can use commercial pannels which are characterized by analyte quantity and produced by accredited manufacturers or accredited laboratory samples containing and not containing nucleotide acids of certain agents in different concentrations, that are stable in proper storage conditions.

In accordance with ISO 22174:2005 "Microbiology of food and animal feeding stuffs – Polymerase chain reaction (PCR) for the detection of food-borne pathogens – General requirements and definitions" and ISO 24276:2006 "Foodstuffs – Methods of analysis for the detection of genetically modified organisms and derived products – General requirements and definitions" several types of control samples are used at different PCR steps:

- *Internal controls* are any DNA samples not similar to DNA of the microorganism to be detected. For tests with which infectious agents are detected sometimes β-globin gene is used, to the needs of which, with the help of gene engineering manipulations, DNA sections are added which are homologous to primers included into the reagent kits.

If internal control DNA is to be included into the reaction together with the sample under analysis then, irrespective of the presence of the microorganism in the biological sample internal control will

become a reason for formation of amplicons which are different in size to specific fragments acquired after PCR in presence of specific DNA of the microorganism.

The presence of internal control amplicons in the reaction will testify to the process of amplification reaction and absence of inhibitors. If amplicons of proper size and internal control amplicons were not formed, one can conclude that there is a problem with PCR: the presence of undesired impurities in the sample analyzed; violation of the technology of reaction. In any case, the result of the reaction would be found invalid.

- *Positive control*, which serves for internal laboratory control over the analysis quality. With the purpose of conducting operative (current) control of the quality of results of laboratory analysis control samples should be included into every kit. This requires DNA sample which contains sites for annealing primers, e.g. DNA of the microorganism to be detected or cloned specific sections of its genome. Non-specific amplicons differ in size from fragments formed as a result of amplification with control DNA sample. They can be both of a larger and smaller size than the positive control. In the worst case their sizes should coincide and be read in the electrophoresis as positive.

For control of the specificity of the formed product of amplification one can use hybridization probes having fluorescent tags or radioactive isotopes and interacting with DNA according to the same principles as primers.

Of special importance is the use of control samples in the following cases:

- a change of a series of reagents;
- a change or recalibrating the amplifier;
- a change of DNA isolation system;
- if automatic determination of the genotype by software is impossible (for amplifiers of DT series).

- *Negative control* includes all reaction components, but instead of clinical material or DNA sample there is a corresponding quantity of deionized water or extract not containing DNA under analysis. Negative control is necessary for verifying reaction components for absence of DNA or agent's cells as a result of contamination and exclusion of false positive results.
- *Special controls* make it possible to estimate the efficiency of amplification process and control the specificity of the obtained results as well as to conduct DNA quantity analysis.

Special controls include the following:

- markers of lengths of DNA fragments;
- background control;
- standards and calibrators;
- control of collecting biomaterial (BMC).

Markers of lengths of DNA fragments are used during detection of results by gel electrophoresis PCR-method. Standards (markers) are fragments of double-strand DNA of an exact length, which make it possible to identify and characterize the bands in the gel and evaluate the analysis results from the point of view of their specificity.

Background control is mostly actual when they the method of hybridization while amplification is applied, as the device registers simultaneously the specific and background fluorescence. The latter's value depends on the properties of marked probes; changes in concentrations of separate components of the reaction depending on the series, mode and storage duration; plastic used; properties of the registering equipment.

Analysis of the target signal value from amplicons above background fluorescence and noises in the process of qPCR makes it possible to set some threshold fluorescence value. It is equal for all simultaneously used samples and is conducted automatically, not requiring additional manipulations on preparation of background samples. However, with the use of FLASH-method during the analysis there is a necessity of introducing separate background tubes.

Standards and calibrators are most often used in conducting quantity analysis by PCR-method. Introduction of this type of controls suggests making a calibration schedule with coordinates with the series of DNA-standard derivations, which is used for detecting concentration of the substance in experimental samples.

The method accuracy depends on the fact how close the conditions of PCR of a series of standards (primarily, amplification efficiency) are to conditions of PCR of the experimental samples.

In cases when one needs to evaluate the "absolute" quantity of the substance, selection of standards for calibration curve is a difficult task.

To determine the matrix quantity in qPCR there are the following variants of standards:

- purified product of qPCR;
- recombinant DNA;
- recombinant RNA with further reverse transcription;
- synthetic oligonucleotide containing an amplified sequence.

Use of standards and calibrators makes it possible to determine the concentration of DNA in two variants (e.g. during analysis for the presence of pathogenic microorganisms in the sample):

- the number of genome equivalents of microorganisms cells per unit of clinical sample volume (GE/ml), which reflects absolute concentration of the given microorganisms in the clinical material;
- calculating the ratio of the number of genomes and the number of genomes of human cells. For this purpose the PCR-mixture, together with calibrators of DNA of the microorganism, there are calibrators of human DNA. Thus obtained relative values of concentration of DNA of the microorganism to human DNA can reflect load density of the microorganisms to be detected.

One should note that ISO 22174:2005 recommends obligatory and scheduled use of control numbered 1 to 7 (Table 1), while controls №8 and 9 are not strictly obligatory. Arrangement of standards is required only for quantity analysis.

Control over collecting material is a key moment in determination of the quality of the sample under analysis. The given approach makes it possible to avoid mistakes at the pre-analytic stage during analysis of the biological material containing human cells and to avoid invalid, false positive or false negative results of PCR. Besides, it can be used for evaluating the quantity of the human genome DNA.

Thus, there is a range of approaches providing for acquiring valid results, which make it possible to control the quality and effectiveness of PCR and leverage the operations of the laboratory.

Precautionary measures during work with PCR Kits

- the kit is to be used in in vitro diagnostics;
- do not exchange the twist-tops of tubes to avoid contamination;
- use only clean tips with a filter and droppers for work with positive control samples;
- tightly close the tops of tubes after using the reagents;
- do not use when expired;
- do not use with reagent kits of other manufacturers.

2. APPROACHES

"DNA-Technology" offers kits for conducting molecular genetic analysis with the use of different technological approaches. Each technology has its peculiarities and field of application. The selected technological platform greatly influences the equipment and organization of the laboratory. Many reagent kits are manufactured in certain variants for various technological platforms.

Depending on the reaction stage, at which results are detected, there can be:

- *End-point PCR* is modification of PCR method when reaction results are considered following all amplification cycles.
- *Real-Time-PCR (qPCR)* is real-time determination of the specific DNA sequence in the sample following each amplification cycle.

Depending on the detection method there can be:

- PCR with electrophoretic detection of results (conventional PCR);
- PCR with fluorescent detection.

2.1. REAL-TIME PCR (QPCR)

At present Real-Time PCR is the most efficient method for solving molecular genetic tasks in the field of diagnostics of infectious diseases, analyzing complex biological systems, such as biocenoses of the urogenital and gastrointestinal tracts, analyzing genetic polymorphisms.

The given method makes it possible to:

- increase the reaction effectiveness;
- decrease the risk of formation of non-specific products;
- provide for the possibility of conducting both quality and quantity analysis of the target DNA/RNA molecule sections.

While developing the PCR kits "DNA-Technology" implements the hydrolyzing probe technology which is based on 5'-exonuclease activity of polymerase.

The reaction mix contain DNA-probes, which include the fluorescent label in 5'-position, fluorescence quencher in 3'-position, as well as the phosphate group in 3'-position. The quencher absorbs the emission of the fluorescent label, while the phosphate group in 3'-position prevents probe from polymerization (Fig. 4).

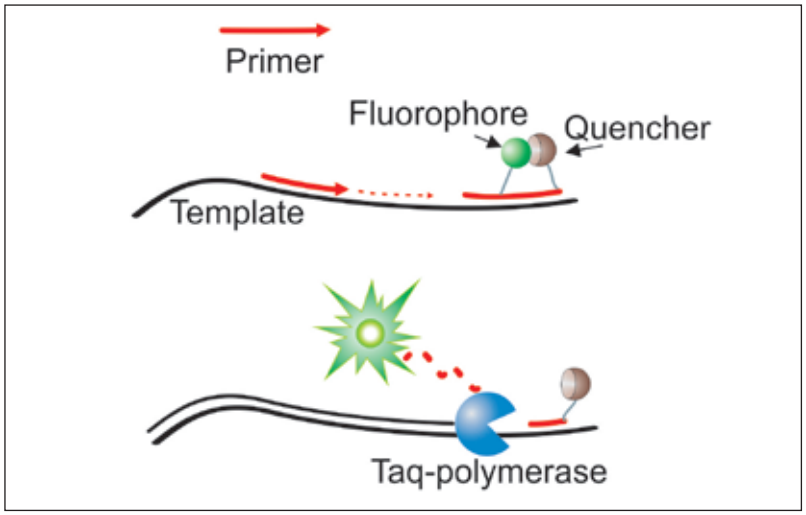

Fig. 4. Principle of the hydrolyzing probe action

During PCR, at the annealing step, DNA-probe hybridize with complementary DNA region, and the more amplification products are formed during PCR, the more probe molecules will connect with corresponding amplicons. At the elongation step polymerase synthesizes DNA complementary strand and following reaching the probe it starts its degradation due to 5'- exonuclease activity.

The advantages of this approach are high signal/noise ratio and high sensitivity of the method.

The real-time analysis requires use of special DNA-amplifiers with optical unit, which make it possible to detect fluorescence inside the reaction tube at each reaction cycle, for example, devices of DT series produced by "DNA-Technology" (DT*lite*, DT*prime*, DT-96, DT-322) (Fig. 5).

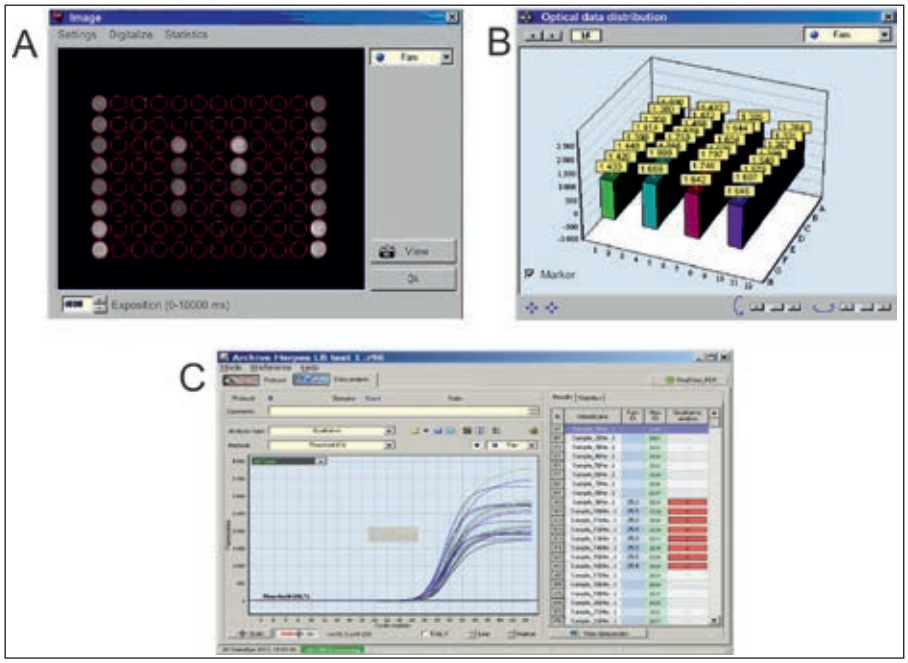

Fig. 5. Analysis of optical measurements with the use of DT*prime* ("DNA-Technology")
A – Fluorescence by Fam channel
B – Data of optical measurements by Fam channel in selected tubes
C – Results of analysis of optical measurements

The given technology is successfully used in PCR Kits *for qualitative and quantitative analysis*, as well as for *analysis of expression of genes*.

During *quantitative analysis* of samples each series of experiments is accompanied by arrangement of amplification with control samples (calibration templates) in which the number of DNA copies is already known. Comparing of kinetics of accumulation of amplification products in experimental and control samples makes it possible to evaluate DNA concentration within the range of DNA control samples.

It should be noted that quantitative PCR requires implementation of DNA samples with a high purification degree as presence of undesired impurities (inhibitors) decreases the effectiveness of amplification of the analyzed and control DNA.

For control of the accuracy we use calibrated internal controls. In some cases there can be losses of DNA at the stage of isolation, which lead to significant distortions the value of real quantity of DNA in the sample. For control over such losses we add to the sample internal control, the quantity of which is determined together with DNA quantity of the infectious agent.

The application field of classic PCR is significantly expanded by:

- multiplex (multiprimer) PCR;
- PCR with reverse transcription (RT-PCR);
- analysis of melting curves.

Multiplex (multiprimer) PCR is simultaneous amplification of two and more DNA sequences in one tube.

PCR with reverse transcription (RT-PCR) is used for identification of known RNA sequence. At the first stage of analysis RNA should be converted to single-strand DNA molecule (cDNA) with an aid of revertase enzyme. At the second stage the acquired cDNA is introduced as a matrix to the standard PCR.

The possibility of use of RNA as a target for PCR significantly widens the application field of this method, for example, genomes of many viruses (hepatitis C, influenza viruses, HIV, etc.) are represented namely by RNA.

Often real-time PCR is combined with RT-PCR for measuring quantity of mRNA, which makes it possible to conclude on the level of expression of a gene in certain tissue.

Analysis of melting curves in systems with DNA-probes provides a possibility of distinguishing point mutations located inside the areas of binding DNA-matrix and probe. Presence of such mutations can lead to changes of probe melting and changes in the melting curve diagram.

During analysis for detecting single-nucleotide polymorphisms as well as short deletions and insertions method allow detection of genotypes: *homozygote of a one kind, heterozygote and homozygote of another kind.*

In implementing the approach of detecting ***single nucleotide polymorphisms (SNPs)*** there is a number of technical peculiarities capable of influencing the quality of the result. The first commercial kits used for analysis of polymorphisms were systems with allele-specific primers and further detection of results in electrophoresis format. A disadvantage of these systems was a high degree of subjectivity during interpretation of results and contamination risk.

The next step in this direction was implementation of intercalating dyes (SYBR-GREEN and analogs). From the point of view of the quality of the results, one should note that use of intercalating dyes significantly more often leads to detection not only of specific sequence, but of non-specific amplification products (for example, primer-dimers), in comparison with similar indices during the use of fluorescent probes.

Besides, test-systems with intercalating dyes suggest using two tubes for detecting one SNP, which reduces the capacity of the laboratory. Another disadvantage is difficulties in interpretation of results obtained with the use of intercalating dyes, from the point of view of automation of this process.

"DNA-Technology" has developed a unique technology of identification of single-nucleotide replacements with the use of PCR followed by melting curve analysis, which makes it possible to accurately and efficiently differentiate the acquired genotypes, automatically interpret the melting curve diagrams and conduct analysis in "single SNP – single tube" format.

During identifying replacements of single nucleotides first they conduct PCR with primers, common for both variants of DNA sequence, then decrease the temperature of the reaction mix for hybridization of the obtained matrix with oligonucleotide probes (Fig. 6).

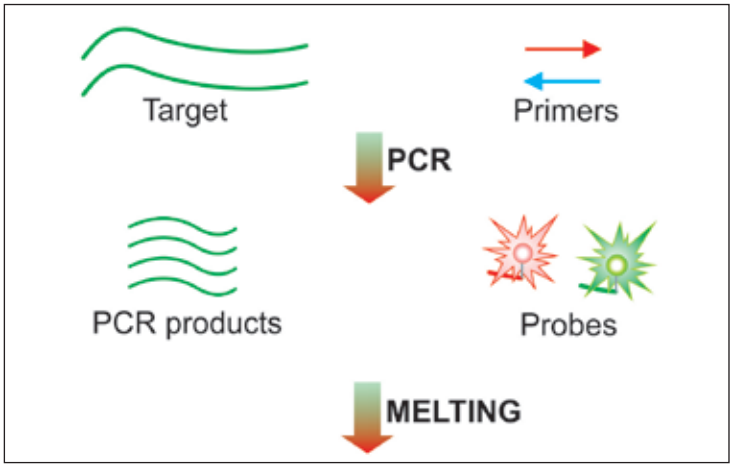

Fig. 6. Molecules involved in PCR and melting processes

In order to implement "hot start" feature to avoid inconvenient physical segregation of polymerase and PCR-mix (i.e. wax layer), prevent non-specific annealing of primers at PCR initial stages and to increase the sensitivity of test-systems, on the whole, "DNA-Technology" offers Taq-polymerase which blocked by specific antibodies.

In order to determine the sequence variant we developed an original modification of *kissing (adjacent) probes or resonance energy transfer*, in the basis of which there is implementation of two types of oligonucleotides *(samples)*, hybridizing to a matrix at a low temperature in close proximity to each other. One of the oligonucleotides is marked with a fluorescent donor, while the other one – with an acceptor (quencher) (Fig. 7).

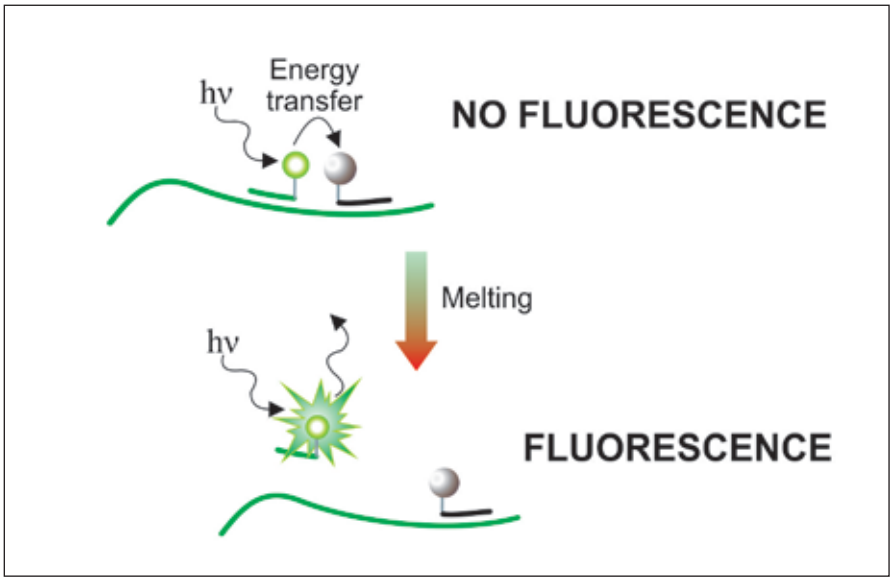

Fig. 7. Implementation of two probes with resonance transfer of energy

In order to identify the nucleotide sequence of the sample analyzed the probes are *melting from the duplexes*, which were formed as a result of hybridization of fragments of DNA oligonucleotide probes, due to consistent increasing of the reaction mix temperature. In order to implement the given approach we use *sequence-specific typing oligonucleotides* and one common oligonucleotide with fluorescence quencher. In order to improve the typing reliability (this is especially important during clinical analysis) we use the method of simultaneous hybridization with two alternative typing probes marked with *different fluorophores*, which makes it possible to determine both variants *in one tube* (Fig. 8).

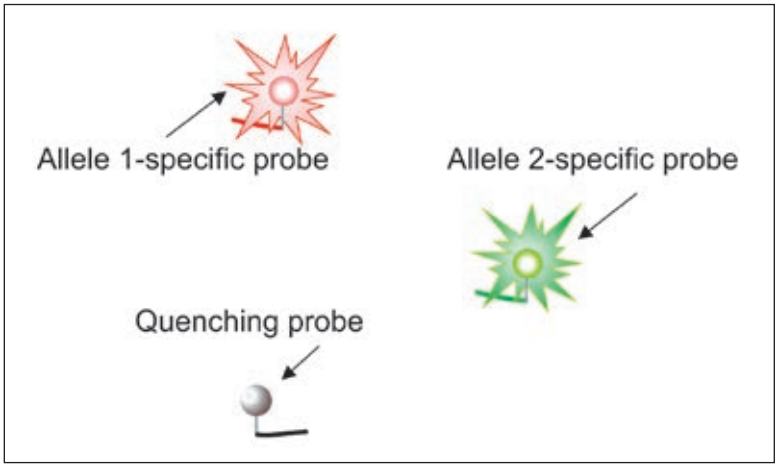

Fig. 8. A mix of oligonucleotide probes

The genotype is determined as a result of measuring the fluorescence level at thermal denaturation of duplexes of oligonucleotides and the matrices obtained. The given measurement is conducted in real-time mode; its result is melting curves.

At the moment of melting of a DNA-duplex, when temperature rises, fluorescence increases, which makes it possible to detect the presence of single nucleotide replacement. If the analyzed fragment has no mutation (normal genotype), the specific "fragment-sample «duplex will melt at higher temperature while the mismatched duplex will melt at lower temperature (Fig. 9).

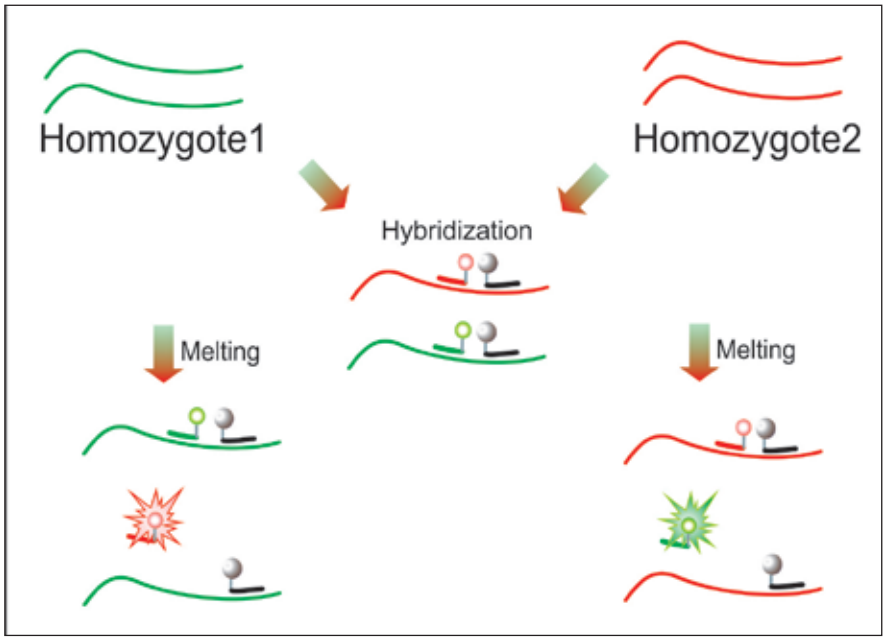

Fig. 9. Hybridization (forming of duplexes) and further melting of homozygous samples

If the analyzed fragment has the mutation (mutant genotype) the high and low temperature melting "fragment-sample» duplexes will demonstrate the opposite pattern of denaturation temperature dynamics.

If we speak about a heterozygote, bearing both types of alleles (mutant and normal) the peaks of melting curves will match (Fig. 10).

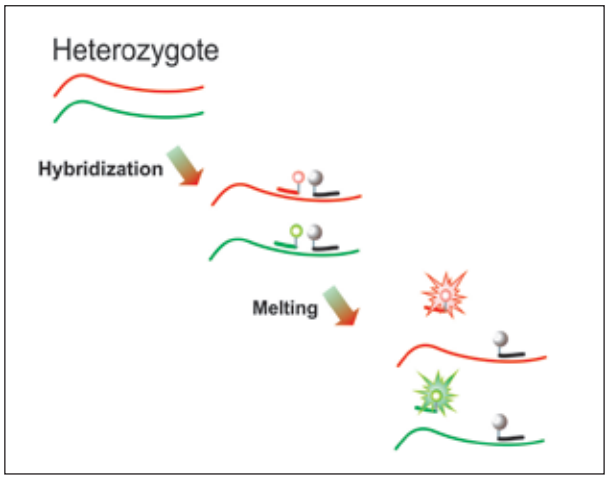

Fig. 10. Hybridization (forming of duplexes) and further melting of heterozygous sample

The described variant of method, thanks to its accuracy, takes leading positions in laboratory practice during scientific research as well, especially with the point of view of SNP analysis by PCR-method.

Taking into account the significance of the analysis of polymorphisms in clinical practice, the main directions of improvement of PCR-method were: automation of the process of treatment of acquired data and giving the result; increasing the accuracy and stability of the system operation. Here we should note a PCR variant with HRM (High Resolution Melting) function, suggested by some manufacturers of reagent kits and equipment for PCR-diagnostics.

In the basis of this approach there is a heteroduplex analysis with implementation of intercaling dyes and further melting of amplicons in one tube. Genotyping is conducted automatically, on the basis of analysis of curve forms, with the use of specialized software. A disadvantage of this approach is a complexity of interpretation of the forms of curves for different genotypes, which causes a necessity of introducing mathematical methods of analysis of the acquired results.

Besides, the difference of melting temperatures for different genotypes (in the cases when it should exist) should amount to 0,1 °C, which makes the system less stable, making impossible reliable automatic genotyping and leaving no possibility for visual interpretation of results.

Implementation of the technology suggested by "DNA-Technology" makes it possible to minimize the interpretation error due to determining the temperature difference for allele variants of not less than 4-5 °C, which provides for maximum stability and reproduction of results. Determining of the melting temperature of oligonucleotide samples is conducted with one device – for example, a detecting amplifier of DT series produced by "DNA-Technology".

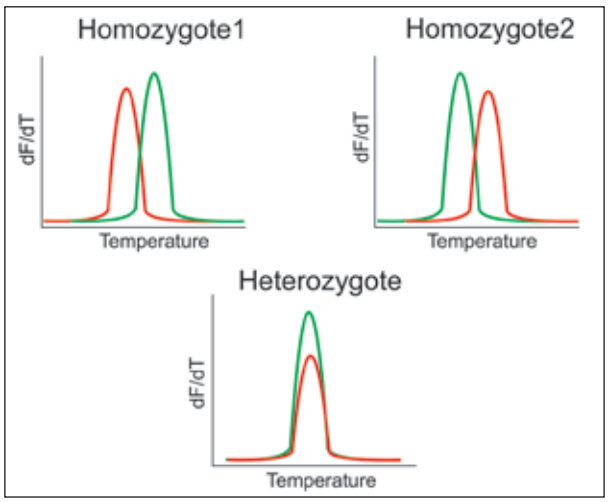

Fig. 11 shows the results of the analysis of individuals with different genotypes by the method of kissing probes with the use of reagent kits by "DNA-Technology" (Fig. 11).

Fig. 11. Melting curves typical for genotyping by the method of kissing probes

2.2. FLASH-PCR

A development of *End-point PCR* was FLASH technology (Fluorescent Amplification-based Specific Hybridization), which is specific hybridization in the process of amplification with DNA-probes (artificially synthesized DNA sections) marked by fluorophores. Instead of electrophoretic detection, it uses fluorescent one. An important benefit of this technology is *possibility of detecting the reaction results without opening the tube*. Thus, it solves one of the main problems of PCR – the problem of contamination with amplicons and, as a consequence, *a probability of obtaining false positive results*. Implementation of the given technological platform provides for less strict requirements to organization of a PCR-laboratory, in particular, it helps to reduce the minimal required number of rooms and involve a less number of personnel.

For the fluorescent detection they use special dyes, intercalating (in-building) in double-strand molecules of DNA, or modified deoxynucleotides, which fluoresce after hybridization with DNA complementary sections.

"DNA-Technology" implements the FLASH technology with the use of *probes with complementary end sequences*. Due to this feature the probe forms stem-loop structure which brings fluorophore and quencher close to each other thus providing effective quenching effect.

During annealing primers the probes complementarily join to the amplified DNA section, the quencher is physically isolated from the fluorophore and the fluorescent signal increases. Such probes are often called molecular beacons (Fig. 12).

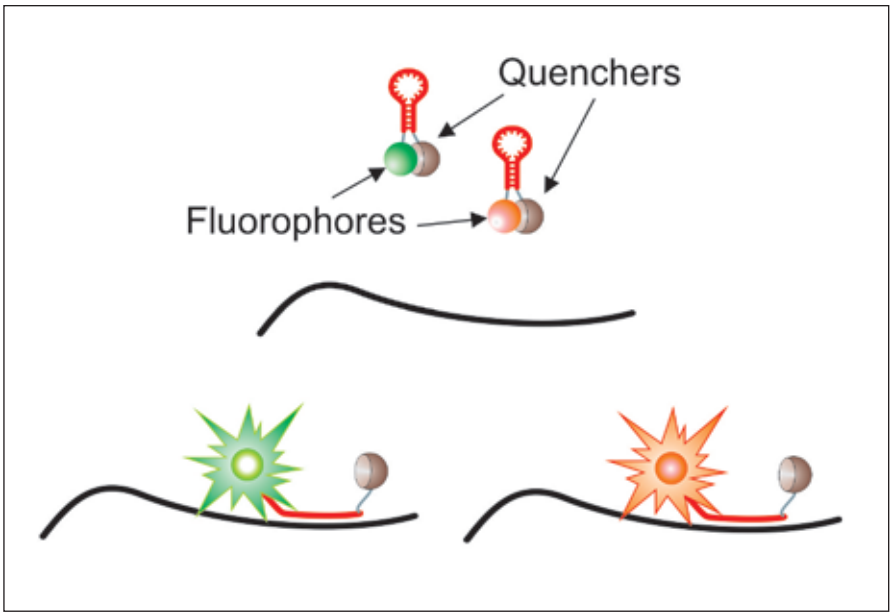

Fig. 12. Probes with complementary end sequences

Thus, the number of joining probes and, correspondingly, the fluorescence level are proportional to the number of formed specific products of PCR. The given approach is characterized with high efficiency and stability of the *probe – detected sequence complex*.

Detection of FLASH-PCR results following the end of amplification reaction requires implementation of special equipment – a fluorescence detector, for example, devices "Gene" or "Gene-4" by "DNA-Technology". In the process of its operation the device registers fluorescent emission produced in the reaction mix when influenced by a source of exciting light. Registration is conducted automatically, in a sequence for each of the tubes (Fig. 13).

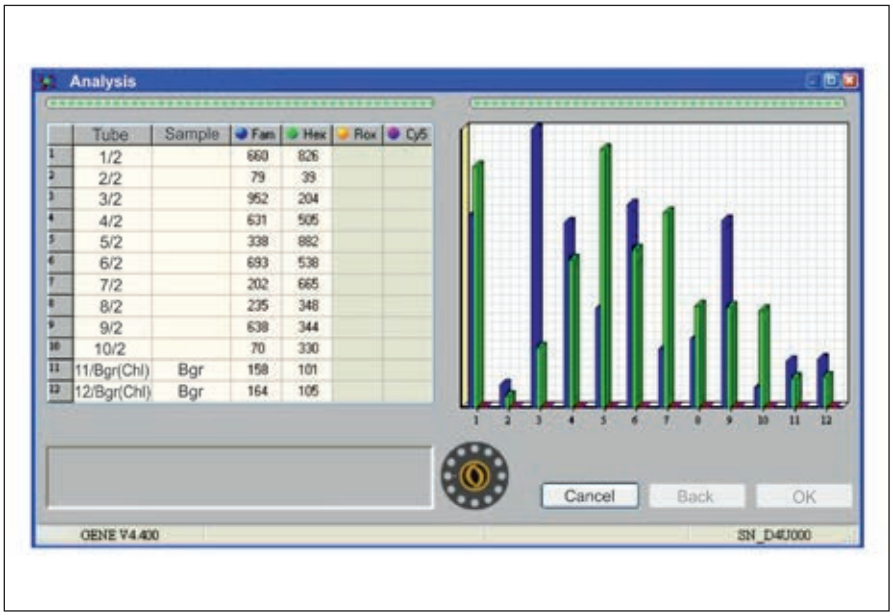

Fig. 13. A result of fluorescence detection by "Gene-4" ("DNA-Technology")

The described detection method significantly reduces the time of the analysis and excludes the possibility of subjective evaluation of the obtained results, which improves the quality of laboratory work. A distinctive feature of the given approach is possibility of *automatic registration and interpretation of obtained results* (Fig. 14).

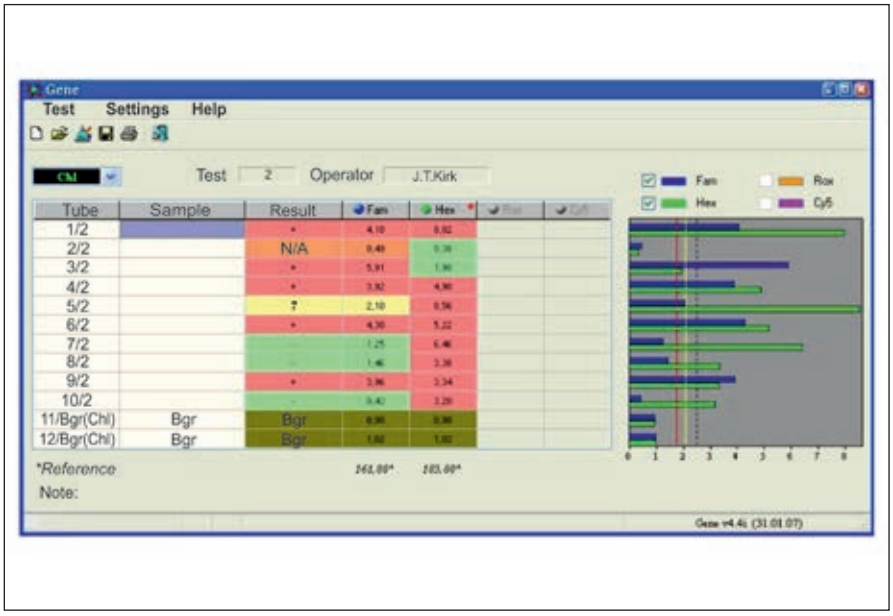

Fig. 14. Analysis of results by "Gene-4" ("DNA-Technology")

Nevertheless, it should be remembered that the given detection method enables *only qualitative analysis.*

2.3. CONVENTIONAL PCR

The given detection variant is used only for *End-point PCR*. The recently most spread electrophoretic method is based on segregation of DNA molecules by size and visualization of the results in the plate of agarose gel, mixed with special coloring agent of DNA, for example, ethydium bromide. The gel plate is placed to the apparatus for horizontal gel electrophoresis and a DC source is connected to it (for example, power supply sources "Elf" by "DNA-Technology").

Negatively charged DNA starts to move in the gel from minus to plus. At that, shorter DNA molecules move faster than longer ones. The movement speed of DNA in the gel is influenced by the following factors: agarose concentration, electric field strength, temperature, composition of electrophoretic buffer and, to a lesser degree, GC composition of DNA. The coloring agents builds-in (intercalates) into DNA molecules.

All molecules of one size move with the same speed. Following the end of electrophoresis, which lasts from 10 minutes to 1 hour, the gel is placed into the transilluminator filter, which emits light in the ultraviolet range (254 – 310 nm). The ultraviolet energy absorbed by DNA in the range of 260 nm, is transferred onto the coloring agent, making it fluoresce in the orange-red area of the visible spectrum (590 nm) (Fig. 15).

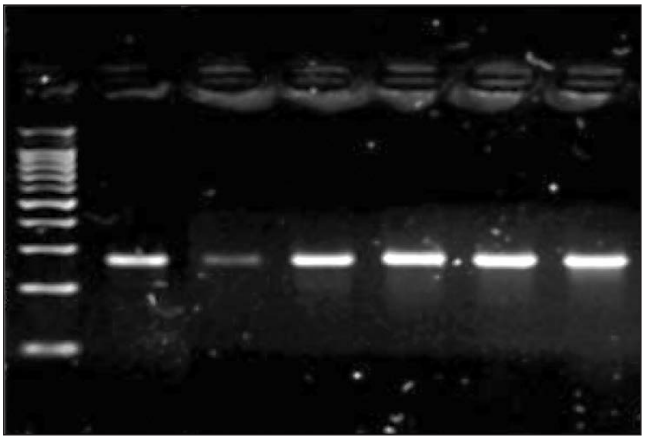

Fig. 15. Results of DNA analysis by the method of gel-electrophoresis (transilluminator photo)

Electrophoretic detection makes it possible to conduct *only qualitative analysis* and is connected with a number of problems:

- large time expenses for the detection stage;
- impossibility of automation;
- complexity and subjectivity of interpretation of results;
- high contamination risk and large expenses for its elimination:
  - increased requirements to organization of the laboratory;
  - maximum distance between the detection area and other PCR areas;
  - a need of a specialist for the detection stage;
  - constant control of wash-outs;
  - great amount of K – for control of contamination with amplicons and, as a consequence, an increase in the amount of expendables and time for preparation for detection.

Successful implementation of the given direction in conditions of clinical diagnostic laboratories requires corresponding equipment and proper organization of the PCR-laboratory.

EQUIPMENT

II. EQUIPMENT

3. THERMAL CYCLERS AND EQUIPMENT FOR PCR PREPARATION

3.1. DT*PRIME* REAL TIME PCR THERMAL CYCLER

CE/IVD

DT*prime* combines innovation of technical solutions, a wide range of settings, design reliability and control ease.

DT*prime* is irreplaceable both in solving diagnostic tasks requiring accuracy, reliability and high capacity and for scientific research, with importance of optimization and adjustment of the analysis parameters.

The device is controlled with the original program DTmaster with Russian and English user interface. Software is common for all amplifiers of DT series with real-time detection of PCR results.

Software makes it possible to use a simple and intuitive control with implementation of "Test" function, which significantly reduces operator's time on creating protocols and minimizes the error probability. Also, the software contains applications for quantity analysis of DNA/cDNA, analysis of melting curves, detecting the level of gene expression, analysis of biocenoses and SNP-analysis, enables the use of the "additional standards" function and create analysis protocols with automatic interpretation of obtained results.

Technical specifications of detecting amplifiers DT*prime* of M and X modification

PARAMETER	VALUE
Design of the thermal plate	One piece unit (non-separated unit) for 96 test tubes 0,2 ml with possibility of setting the gradient in two directions (12x8 format, M1 modification) One piece unit (non-separated unit) for 384 test tubes 0,045 ml (24x16 format, modification X1) There can be the following design variants of the thermal unit work surface: 1) 3-sectional module for 96 test tubes 0,2 µL with possibility of adjusting the temperature difference between sections and gradient in one direction (12x8 format, modification M3) 2) 6- sectional module for 96 test tubes 0,2 with possibility of adjusting the temperature difference between sections, with no possibility of adjusting the gradient (12x8 format, modification M6)
Tube type	For M modification devices: • Standard microtubes 0,2 ml for real-time PCR • Strips 8x0,2 ml for real-time PCR • Microplate 96x0,2 ml for real-time PCR For X modification devices: • Microplate 384x0,045 ml for real-time PCR
Maximum volume of reaction mix, ml	0,1 (for M modification devices) 0,03 (for X modification devices)

Tube height	Tube height automatic adjustment
Temperature range of the thermal unit	0...100 °C
Temperature increment	0,1 °C
Precision of temperature maintenance	±0,2 °C
Temperature irregularity of thermal unit	±0,15 °C
Heating ramping rate, average (maximal)	3.3 °C /sec (for M modification devices) 2,1 °C /sec (for X modification devices)
Cooling ramping rate, average (maximal)	2.1 °C /sec (for M modification devices) 1,0 °C /sec (for X modification devices)
Active heating/cooling device of the thermal unit	Peltier elements
Thermal block lid heating (maximal)	105 °C
Thermal block draw-out	Automatic
Device calibration	Default (settings provided as requested)
Possibility of simultaneous detection of amplification products (detection channels)	4 (modification DT*prime* 4) 5 (modification DT*prime* 5)
Excitation sources	LED per each channel
Detector	CCD camera
Spectral range	470 nm – 675 nm for devices with 4 detection channels (DT*prime* 4) 470 nm – 750 nm for devices with 5 detection channels (DT*prime* 5)
Power supply network voltage, V	220
Power supply network frequency, Hz	50
Dimensions (width x length x height), mm	210 x 540 x 540
Weight, kg	27

Advantages of DT*prime*:

- High performance thanks to a wide range of thermal blocks in 96 and 384 wells format adapted to microplates, tubes and strips.
- Up to 5-channel multiplex fluorescence detection for a variety of fluorescence dyes and phluophlore combinations in a wide spectral range. The standard set of DT*prime* is supplied with 4 channels set for detection of Hex, Fam, Cy5, Rox dyes. (It is possible to install a filter for fluorescence detection in the range of Cy5,5).
- Light emitting diodes (LED) as a light source that, unlike halogen lamps, does not deteriorate over the life-time of about 100,000 hours.
- High sensitivity, optimal signal/noise ratio and a low interchannel crosstalk ensured by the unique design of the optical track including a separate light source for each channel and a matrix CCD camera.
- Broad dynamic range of detection achieved by employing multiple exposure method, which takes the optimization of signal registration conditions to a whole new level, greatly simplifying or even eliminating the need for photometric settings.

- Optical systems with various spectrum parameters enable operation with multiple fluorophlores.
- The thermal block makes it possible to set the gradient of annealing and/or thermal block temperature difference, which opens additional possibilities for optimizing amplification programs.
- Keeping regular thermal block temperature, which provides for high reliability and replication of results.
- The device has protection from interruptions of network power supply (automatic resuming of amplification program following the restoration of network power supply)
- The device has protection from computer failures (automatic resuming of device operation in case of any problems with the computer (up to its replacement) with automatic reading of results of optical measurements from the device to computer following their elimination)
- Realized is the possibility of operator's diagnostics of the thermal cycling system and the device optical system directly in the process of amplification program running
- Provided for is the possibility of work with the program both in the compact and extended modes, which provides for comfortable environment for clinical and research applications.
- The possibility of work with robotized sample preparation systems thanks to special design of the body and automatically-operated draw-out thermal block.
- Possibility of being easily integrated with any laboratory information system (LIS) as the device can save all data in a standard graphic or text formats ready to be loaded into databases.
- Several devices can be controlled simultaneously by one computer. Moreover any batch of data can be viewed and analyzed by the user using computer while another amplification program is being executed.
- The compact body design, allowing several devices to be placed closer together to increase productivity at minimal space requirements.
- The device is an open system and is not limited for use with specific reagents or plastic. At the same time "DNA-Technology" supplies test systems for the majority of real time PCR applications that exist today.

3.2. DT*lite* REAL TIME PCR THERMAL CYCLER

CE/IVD

DT*lite* is innovational solutions in a compact body.

DT*lite* is irreplaceable for diagnostic laboratories with low and medium pass-through capacity. The device is adapted both for routine diagnostics and scientific research, with possibility of optimization and fine adjustment.

The device is controlled by DT*master* software with Russian/English user interface, common for all amplifiers of DT series with detection of results of real-time PCR.

The software makes it possible to use easy and intuitive control with "Test" function, which significantly reduces operator's time expenditure on creating protocols and minimizes the error probability. Also, the software contains applications for quantity analysis of DNA/cDNA, analysis of melting curves, detecting the level of gene expression, analysis of biocenoses and SNP-analysis, enables the use of the "additional standards" function and create analysis protocols with automatic interpretation of obtained results.

Technical Parameters of DT*lite* detecting amplifiers of L and S modification

PARAMETER	VALUE
Design of the thermal plate	2-sectional module for 48 test tubes 0,2 µL with possibility of adjusting the temperature difference between sections (8x6 format, modification S1) One piece unit (non-separated unit) for 192 test tubes 0,045 ml (16x12 format, modification L1)
Tube type	For S modification devices: • Standard microtubes 0,2 ml for real-time PCR • Strips 8x0,2 ml for real-time PCR For L modification devices: • Microplate 192x0,045 ml (half of a standard microplate 384x0,045 ml) for real-time PCR
Maximum volume of reaction mix, ml	0,1 (for S modification devices) 0,03 (for L modification devices)
Tube height	Tube height automatic adjustment
Temperature range of the thermal unit	0°... 100 °C
Temperature setting increment	0,1 °C
Precision of temperature maintenance	±0,2 °C
Temperature irregularity of thermal unit	±0,3 °C
Heating ramping rate, average (maximal)	5 °C / sec (for S modification devices) 3,5 °C / sec (for L modification devices)
Cooling ramping rate, average (maximal)	2,5 °C / sec (for S modification devices) 1,5 °C / sec (for L modification devices)

Active heating/cooling device of the thermal unit	Peltier elements
Thermal block lid heating (maximal)	105 °C
Thermal block draw-out	Automatic
Device calibration	Default (settings provided as requested)
Possibility of simultaneous detection of amplification products (detection channels)	4 (modification DT*lite* 4) 5 (modification DT*lite* 5)
Excitation sources	LED per each channel
Detector	CCD camera
Spectral range	470 nm – 675 nm for devices with 4 detection channels (DT*lite* 4) 470 nm – 750 nm for devices with 5 detection channels (DT*lite* 5)
Power supply network voltage, V	220
Power supply network frequency, Hz	50
Dimensions (width x length x height), mm	210 x 480 x 310
Weight, kg	17

Advantages of DT*lite*:

- High performance thanks to a wide range of thermal blocks in 48 and 192 wells format adapted to microplates, tubes and strips.
- Up to 5-channel multiplex fluorescence detection for a variety of fluorescence dyes and fluophlore combinations in a wide spectral range. The standard set of DTlite is supplied with 4 channels set for detection of Hex, Fam, Cy5, Rox dyes.
- Light emitting diodes (LED) as a light source that, unlike halogen lamps, does not deteriorate over the life-time of about 100,000 hours.
- High sensitivity, optimal signal/noise ratio and a low interchannel crosstalk ensured by the unique design of the optical track including a separate light source for each channel and a matrix CCD camera.
- Broad dynamic range of detection achieved by employing multiple exposure method, which takes the optimization of signal registration conditions to a whole new level, greatly simplifying or even eliminating the need for photometric settings.
- Optical systems with various spectrum parameters enable operation with multiple fluorophlores.
- The thermal block makes it possible to set the gradient of annealing and/or thermal block temperature difference, which opens additional possibilities for optimizing amplification programs.
- Keeping regular thermal block temperature, which provides for high reliability and replication of results.
- The device has protection from interruptions of network power supply (automatic resuming of amplification program following the restoration of network power supply)
- The device has protection from computer failures (automatic resuming of device operation in case of any problems with the computer (up to its replacement) with automatic reading of results of optical measurements from the device to computer following their elimination)
- Realized is the possibility of operator's diagnostics of the thermal cycling system and the device optical system directly in the process of amplification program running

- Provided for is the possibility of work with the program both in the compact and extended modes, which provides for comfortable environment for clinical and research applications.
- The possibility of work with robotized sample preparation systems thanks to special design of the body and automatically-operated draw-out thermal block.
- Possibility of being easily integrated with any laboratory information system (LIS) as the device can save all data in a standard graphic or text formats ready to be loaded into databases.
- Several devices can be controlled simultaneously by one computer. Moreover any batch of data can be viewed and analyzed by the user using computer while another amplification program is being executed.
- The compact body design, allowing several devices to be placed closer together to increase productivity at minimal space requirements.
- The device is an open system and is not limited for use with specific reagents or plastic. At the same time "DNA-Technology" supplies test systems for the majority of real time PCR applications that exist today.

3.3. TERCYC PCR THERMAL CYCLER

Four-channel DNA-amplifier Tercyc is designed for amplification in tubes 0,5 ml. The device is a single module combining 4 independently controlled thermal units.

Technical Parameters

PARAMETER	VALUE
Number of independent thermal units, pcs	4
Number of tubes 0,5 ml in a thermal unit, pcs	10
Operating volume of the reaction mix, µL	10–50
The temperature regulation range, °C	4...99
Precision of temperature maintenance, °C	+/– 0,3
Dynamic regulation error, °C	+/– 0,3
Non-identity of thermal blocks (maximal), °C	0,2
Cycle duration (92 °C – 1 sec, 72 °C – 1 sec) for 15 µL, sec	64
Number of programs	up to 28
Power consumption at 220 V (maximal), W	95
Dimensions (width x length x height), mm	240 x 210 x 80
Weight, kg	4

Advantages of Tercyc:

- Each thermal unit can hold up to 10 test tubes with the volume of the reaction mix ranging from 10 to 50 µL;
- the device supports three methods of regulating the temperature of a reaction mix:
  - passive (based on the temperature of the unit);
  - two active methods (a mathematical model);
- the LCD display allows for programs to be entered and for thermocycling to be controlled and operated without the need for a regular PC;
- the device can work with any IBM compatible PC;
- low variation of temperature within a thermal unit;
- noiseless operation.

The quality of the reaction is improved significantly due to more accurate conditions for primer annealing, ensuring high activity of the Taq DNA polymerase and reducing the accumulation of non-specific amplification products.

Independent channels make it possible:

- to perform several assays simultaneously;
- have several users operating the device at the same time;
- expedite the optimization of new reaction conditions;
- treat up to 40 samples using the same program regimen during large-scale tests.

3.4. GENE FLUORESCENCE DETECTOR

The device is intended to record the results of PCR using sets of reagents, based on the principles of fluorescence detection (*end-point PCR*).

Minimal requirements to the controlling computer for the detector "Gene":

- operating system from Windows-98 and above
- Pentium I processor with a clock frequency of 200 MHz
- RAM at least 32 MB
- the amount of free disk space for at least 10 MB
- CD drive
- minimum screen resolution of 1024x768 pixels.

Technical specifications

PARAMETER	VALUE
Number of detection channels	2
Wavelengths of excitation / emission, nm	470 / 514 532 / 580
Time detection of one block (12 tubes), sec	max. 30
Power consumption of the device (from a computer through a USB port)	5V, max. 500 mA
Light excitation source	LED
Light detector	Photodiod
Detector sensitivity limit in 30 ml of reaction mix, pmol/ simultaneously	0,001
Number of tubes in the rotor	12
Supply voltage, V	220
Supply frequency, Hz	50
Dimensions LxWxH, mm	240 x 150 x 90
Unit weight, kg	1,3

The advantages of the Gene Fluorescence Detector:

- recording of the results of PCR without electrophoresis (without opening the tubes), allows to solve the problem of contamination with PCR products and allows you to reduce the demands on the organization of a PCR laboratory;
- the absence of electrophoresis stage significantly reduces the complexity and time of the result;
- performance allows you to organize large flows of routine laboratory studies;
- the software that came bundled with the device allows you to effectively organize documentation and work with the results obtained;
- small size.

The instrument focuses on the use of probes and / or primers labeled with a fluorescent label and quencher of fluorescence.

3.5. GENE-4 FLUORESCENCE DETECTOR

CE/IVD

The device is intended to record the results of PCR using sets of reagents, based on the principles of fluorescence detection.

Technical specifications

PARAMETER	VALUE
Number of detection channels	4
Wavelengths of excitation / emission, nm	460 / 520 532 / 570 580 / 620 632 / 675
Time detection of one block (12 tubes), sec	30
Power consumption of the device (from a computer through a USB port)	5V, max. 500 mA
Light excitation source	LED
Light detector	Photodiod
Detector sensitivity limit in 30 ml of reaction mix, pmol/ simultaneously	0,002
Replaceable rotors for tubes 0,5 and 0,2 ml	2
Number of tubes in the rotor	12
Supply voltage, V	220
Supply frequency, Hz	50
Dimensions LxWxH, mm	240 x 200 x 110
Unit weight, kg	1,4

The advantages of the Gene-4 Fluorescence Detector:

- The possibility to use probes and/or primers with fluorescent markers and quenchers;
- Reduced labor costs and the time it takes to get results;
- Closed-tube format for fluorescence analysis of PCR products (no electrophoretic analysis) minimizes the risk of in-laboratory contamination with PCR products and sets less stringent requirements for PCR laboratory organization;
- USB interface/power supply;
- Replacement rotor for 0.5/0.2-ml microtubes;
- The efficacy of the device makes it possible to organize routine laboratory tests on a large scale;
- The software for effective results processing, documentation and reporting;
- Compact design.

The instrument focuses on the use of probes and / or primers labeled with a fluorescent label and quencher of fluorescence.

3.6. ELF-4 POWER SUPPLY

Elf-4 Power Supply is designed for electrophoresis of nucleic acids and proteins in agarose and acrylamide gels.

Technical specifications

PARAMETER	VALUE
Output voltage, V	from 5 to 400
Output current, mA	from 5 to 400
Output power, W	from 0,5 to 80
Timer range	from 1 min to 16 h
Dimensions, mm	180 x 120 x 60
Locks	Short circuit Open circuit Earth leakage Sudden change in load Internal testing

Features of the Elf-4 power supply:

- preferable to agarose gels;
- capable of operation voltage regulation, current stabilization or stabilization of power;
- a built-in timer allows you to disable the electrophoresis after a specified period of time, which prevents the loss of the results of electrophoresis due to excessive distillation.

3.7. ELF-8 POWER SUPPLY

Elf-8 Power Supply is designed for electrophoresis of nucleic acids and proteins in agarose and acrylamide gels.

Technical specifications

PARAMETER	VALUE
Output voltage, V	from 10 to 800
Output current, mA	from 3 to 200
Output power, W	from 0,5 to 80
Timer range	from 1 min to 16 h
Dimensions, mm	180 x 120 x 60
Locks	Short circuit Open circuit Earth leakage Sudden load change Internal testing

Features of the Elf-8 Power Supply:

- preferable for acrylamide gels;
- capable of operation voltage regulation, current stabilization or stabilization of power;
- a built-in timer allows you to disable the electrophoresis after a specified period of time, which prevents the loss of the results of electrophoresis due to excessive distillation.

3.8. PCR-CABINET

The PCR box/cabinet is designed for organizing a space in a clinical, diagnostic or PCR laboratory that is isolated from the external environment.

Technical specifications

PARAMETER	VALUE
Dimensions, mm	1220 x 672 x 670
Maximal distance the front panel can be moved up, mm	370
Number of 220-volt power outlets	3
Maximum total current of connected equipment (220 V), A	10
Power consumed by fluorescent and ultraviolet lamps, W	30
UV-radiation locks	When the front panel is open When the light is switched on
Built-in UV radiation timer	Yes

The advantages of the PCR-cabinet:

- The front glass panel smoothly slides up and can be fixed in position at any height;
- an embedded set of AC power outlets allows laboratory equipment to be connected to power inside the box;
- an antibacterial exciter controlled by an electronic timer;
- ultraviolet radiation is blocked when the front glass panel is opened and when the PCR box is switched on;
- the body is made from coated stainless steel and glass, which prevents corrosion when the surface is treated with aggressive detergents (hydrochloric acid, chlorine based and other types of solutions);
- the body is made with high precision lasers from one sheet of stainless steel to reduce the number of seams and gaps and to ensure effective radiation and treatment of the internal surfaces with detergents;
- the working space is 1200 x 600 mm which makes it possible to have a full-fledged working place inside the PCR box;
- the lighting of the work desk is sufficient for high precision work; convenient layout and ease of operation make the box indispensable when carrying out large numbers of routine laboratory tests.

3.9. THERMIT THERMOSTAT

The Thermit Thermostat is a solid state thermostat for research and clinical diagnostic studies. It is designed for use with 1.5- and 0.5-ml microfuge test tubes.

The Thermit Thermostat is the best choice when the incubation temperature doesn't have to be changed often during operation.

Technical specifications

PARAMETER	VALUE
Number of 1,5 ml Eppendorf test tubes, pcs	40
Number of 0,5 ml Eppendorf test tubes, pcs	28
Temperature range, °C	From room temperature to 99 °C
Countdown timer	From 1 minute to 99 hours
Precision of temperature maintenance, °C	±1
Increment of temperature setting, °C	1
Power consumption at 220 volts, W Initial heating, W Maintaining a temperature, W	200 max. 200 max. 40
Dimensions, mm	250 x 120 x 80
Weight, kg	1,5

The advantages of the Thermit Thermostat:

- a built-in timer;
- compactness;
- low profile of the thermoblock, which creates additional convenience for a laboratorian;
- several thermostats can be comfortably positioned next to each other.

3.10. GNOM PROGRAMMABLE THERMOSTAT

The Gnom Programmable Thermostat is a programmable solid state heating unit for research and clinical diagnostic studies. It's designed to use 1.5- and 0.5-ml test microtubes.

The Gnom Programmable Thermostat proves especially handy when using regimens comprising several stages with different incubation time-temperatures as well as when heating test tubes at high temperatures.

Technical specifications

PARAMETER	VALUE
Number of 1,5 ml Eppendorf test tubes, pcs	40
Number of 0,5 ml Eppendorf test tubes, pcs	28
Temperature range, °C	From room temperature to 99 °C
Countdown timer	From 1 minute to 99 hours
Precision of temperature maintenance, °C	± 0,5
Increment of temperature setting, °C	1
Power consumption at 220 volts, W Initial heating, W Maintaining a temperature, W	200 max. 200 max. 40
Dimensions, mm	195 x 185 x 125
Weight, kg	2

The advantages of the Gnom Programmable Thermostat:

- the built-in programmability allows the device to implement incubation regimens that include one to three consecutive time-temperature intervals;
- the device has a heat insulated clamp lid that improves the distribution of temperature within the unit, prevents the test tube caps from opening at high temperatures and reduces the condensation of the reaction mix on the caps;
- a built-in fan significantly reduces the cooling time;
- an LCD display makes controlling the device easy and comfortable.

3.11. DT*PACK* MICROPLATE HEAT SEALER

The device uses various types of microplates and films. The flexibility of settings and a compact design make DT*pack* Microplate Heat Sealer useful and necessary for conducting analysis with implementation of microplates for real-time PCR.

The device is efficient for using microplates of various types and designs, height and materials, of various manufacturers, including PCR-plates with "skirts", "semi-skirts" and without "skirts". Sealing parameters, including optimal block temperature heating, pressing force of the movable carriage and the time, can be selected with implementation of special software and stored in the inbuilt memory of the device.

The LCD-display shows the device state, the selected parameters, including the current temperature and the sealing time. Thanks to the possibility of selecting parameters optimized for combinations of certain plates and films, DT*pack* Microplate Heat Sealer ensures repeatability and reliable sealing, preventing sample losses caused by insufficient or excessive sealing.

Technical specifications

PARAMETER	VALUE
Supply voltage, V	220
Supply frequency, Hz	50
Work surface temperature, °C	100–200
Absolute error of temperature maintaining, °C	± 2
Sealing time, sec	0,1–9,9
Max. pressing force, kg	12
USB	2,0
Device weight, kg	4,5
Dimensions (LxWxH), mm	315 x 244 x 190

The advantages of DT*pack* Microplate Heat Sealer:

- easy 2-button control making possible sealing of a microplate in four steps;
- compatibility with various plates and sealing films;
- replacement blocks for 96- and 384-well PCR-plates (blocks for plates, adapters and tools of any form can be custom-made);
- optimizable and controllable sealing process;
- electric drive with regulated pressing force;
- USB-interface for programming sealing parameters;
- regulated temperature, sealing time and pressing force for forming profiles of optimal operating parameters;
- in-built memory for storing up to four optimized profiles;
- coding on the LCD-screen with the use of signs and symbols for informing about the current state and selected sealing parameters;
- automatic waiting mode for quick restart and energy saving;
- ergonomic design.

"DNA-Technology" offers the following equipment for PCR-analysis

NAME	CATALOGUE NUMBER*
DT*prime* Real-Time Thermal Cycler 4M1 modification (*4 channels; 96 x 0,2 ml*), with software	O-DTPRIME4M1
DT*prime* Real-Time Thermal Cycler 5M1 modification (*5 channels; 96 x 0,2 ml*), with software	O-DTPRIME5M1
DT*prime* Real-Time Thermal Cycler 5M3 modification (*5 channels; 3-sectional thermal block; 96 x 0,2 ml*), with software	O-DTPRIME5M3
DT*prime* Real-Time Thermal Cycler 5M6 modification (*5 channels; 6- sectional thermal block; 96 x 0,2 ml*), with software	O-DTPRIME5M6
DT*prime* Real-Time Thermal Cycler 5X1 modification (*5 channels; thermal block 384 x 0,1 ml*), with software	O-DTPRIME5X1
DT*lite* Real-Time Thermal Cycler 4S1 modification (*4 channels; 48 x 0,2 ml*), with software	O-DTLITE4S1
DT*lite* Real-Time Thermal Cycler 5S1 modification (*5 channels; 48 x 0,2 ml*), with software	O-DTLITE5S1
Tercyc PCR Thermal Cycler	O-TP4
"Gene" Fluorescence Detector	O-GENE
"Gene-4" Fluorescence Detector	O-GENE4
GnomProgrammable Thermostat	O-TT1
Thermit Thermostat	O-TT2
PCR cabinet	O-BOX/01
Device for electrophoresis of nucleic acids in agarose and acrylamide gels Power Supply Elf-4 (*programmable to 400 V*)	O-ELF4
Device for electrophoresis of nucleic acids in agarose and acrylamide gels Power Supply Elf-8 (*programmable to 800 V*)	O-ELF8
DT*pack* Microplate Heat Sealer	O-DTPACK

* On the ordering and pricing information on the products inquire the sale department.

3.12. GUARANTEE AND POST-GUARANTEE SERVICE

During the guarantee period the company provides guarantee repair works and maintenance of the equipment. After the expiration of this period post-guarantee service of devices is provided.

Service engineers install devices in PCR-laboratories and provide training on operation of the equipment.

Organization and Establishment of a PCR-laboratory

4. ORGANIZATION AND ESTABLISHMENT OF A PCR-LABORATORY

4.1. ORGANIZATION OF A PCR- LABORATORY

At present, organization of a PCR- laboratory is regulated by **ASTM E1873 – 06** «Standard Guide for Detection of Nucleic Acid Sequences by the Polymerase Chain Reaction Technique», 2006 and recommended by WHO «Establishment of PCR laboratory in developing countries», 2011.

A PCR laboratory should contain two functional work areas: a ***pre-amplification*** area and a ***post-amplification*** area (fig. 16). These two areas should ideally be in separate rooms, or when space constraints exist, separate work stations/biosafety cabinets in a single room. Supplies and equipment should be dedicated to each work area and should not be interchanged between areas.

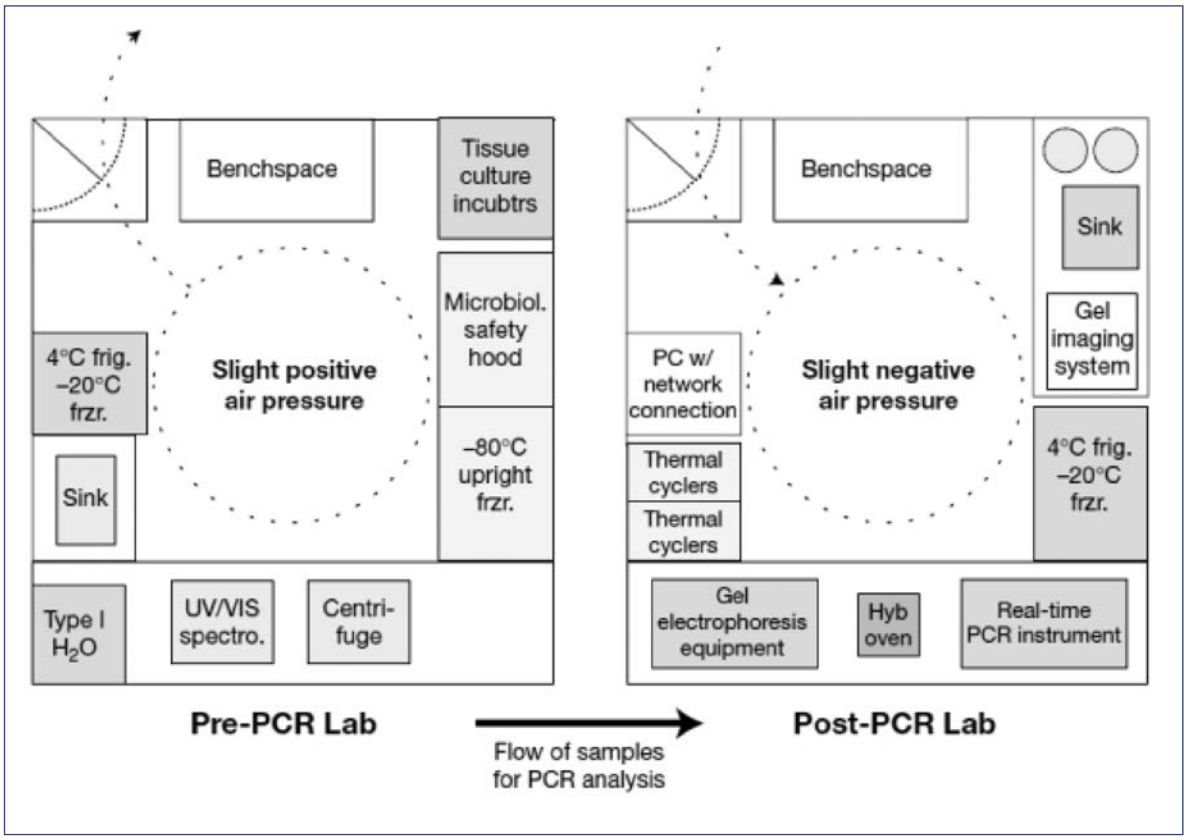

Fig. 16. PCR laboratory organisation (Setting Up a PCR Laboratory. Theodore E. Mifflin. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA, 2003)

The main source of contamination is the amplicons generated during the PCR reactions. Therefore by separating the PCR area («post-PCR») from the PCR preparation area («pre-PCR») the potential for contamination is significantly reduced. The most appropriate condition if there are separate rooms where these activities occur. Miffin (2007) outlines a possible separation of the pre-PCR and post-PCR laboratories in a way that the «forward flow» concept is implemented. Moreover, different air pressures inside the two laboratories decrease the risk of cross-contamination. In the sample preparation room the pressure should be higher, while in the post PCR room it should be slightly reduced. It is ideal if each separated area has its own air supplier. For further reduction of the risk of contamination it is recom-

mended to maintain the doors closed in all rooms of the PCR laboratory. Besides these requirements it is important to supply the separated PCR laboratory areas with equipments, devices and reagents which are used only in the allotted room.

For the prevention or reduction of potential contamination during the PCR detection of special nucleic acid sequences the unidirectional workflow must be applied in the molecular laboratory. It means that during the different work phases the steps of the analysis have to pass from the clean (pre-PCR) to the dirty (post-PCR) areas (referred to as forward flow).

The closed-tube system represent an additional safe mode against cross-contamination. In this case the reaction tubes are not opened after the PCR processing, thus reducing the risk of contamination of the molecular laboratory by the amplicons; moreover elimination of laborious post-PCR sample Real Time PCR representing a closed tube system, therefore it is less sensitive for the cross-contamination than the conventional PCR.

The *ISO 22174:2005* summarises the requirements for PCR-based molecular techniques used for the detection of microorganisms in food samples. For the organisation of a PCR laboratory and for the sample handling the standard recommends the «forward flow» principle and the systematic containment of the methodological steps involved in the production of the results. By keeping these measures it is ensured that the DNA from the test sample and the amplified PCR product remain physically separated during the detection procedure. For this purpose it is recommended that *minimum four distinct areas with their own working facilities* should be separated:

- The first area should be a laboratory for *nucleic acid preparation* from the test material.
- The second area should be the work area *for master mix preparation*, where all the reaction components necessary for the PCR amplification (except nucleic acid) are mixed together.
- The third area serves for the *addition of the separated nucleic acid* to the reaction mixture
- The fourth area is for the *detection and confirmation of PCR products*.

The PCR thermocycler can be placed in the third or in the forth work area, and so the amplification step has been separated from the nucleic acid extraction and from the master mix preparation.

The ideal situation is if all the four work areas are separated physically as distinct rooms and the pre-PCR and post-PCR areas have slightly increased and decreased air pressures, respectively.

4.1.1. SAMPLE PREPARATION

The nucleic acid nalysis starts with the sample preparation. The *ISO 20837:2006* document provides criteria for producing samples which are compatible with PCR and for separation of nucleic acid suitable for PCR analysis. This area has to have its own devices which should not leave the room. In sample preparation area positive-displacement pipettes or pipettors with aerosol-resistant tips, disposable, powder-free gloves and laboratory coats assigned to that room are suggested to use. Fresh gloves and laboratory coats should be worn at all times to control contamination from this room to any other location.

This facility should be used for aliquoting of sample and preparation of positive and negative controls. As per the protocol used for extraction of nucleic acid the required quantity of clinical sample should be added to lysis buffer and then transferred to RNA /nucleic acid extraction area. The processed samples and controls are then added to tubes containing PCR master mix in this room. PCR tubes should be capped as soon as the sample is added.

This room should be kept under negative pressure to prevent escape of infectious agent outside of the room. Biosafety cabinet (for pathogens), refrigerator, freezer and dry heat block/water bath are needed to be placed in this area. Reagents and solid items destined for the sample preparation room have to be autoclaved separately from the other reagents and materials.

4.1.2. PREPARATION OF THE REACTION MIXTURE

For preparation of reaction mixture which contains all the necessary reagents for the nucleic acid amplification filter tips, micropipettes, positive–displacement pipettes or pipettors, amplification reagent, appropriate supplies, PCR-cabinet, glows and dedicated laboratory coats are needed. The reagents have to be kept in fridge or in freezer, thus these apparatuses are also important equipments of this area. By applying a PCR-cabinet (PCR workstation) equipped with UV irradiation it is possible to have a safe, nucleic acid-free environment which minimises the potential for PCR reaction contamination, since the UV light destroys the contaminating DNA inside the cabinet.

To prevent cross-contamination and to avoid repeated freezing and thawing, reagent-stock solutions should be aliquoted into smaller volumes. To deter contamination, theroom should be under positive pressure.

Personnel should complete tasks in this room before working in the sample processing or amplification/detection rooms and should not return from these rooms to the reagent preparation room.

4.1.3. ADDITION OF NUCLEIC ACID TO THE REACTION MIXTURE

For this step a clean area is recommended. If there is no place in the molecular laboratory for separating a dedicated room for nucleic acid addition, this procedure can be made in the area of sample preparation. It is essential that handling of post-PCR materials is not allowed in this part of the room. The amplification is preferably carried out in this room, or if it is not possible (no place is available) it can be done in the area of detection and confirmation of PCR amplified nucleic acid.

4.1.4. DETECTION AND CONFIRMATION OF PCR AMPLIFIED NUCLEIC ACID

The PCR thermocycler has to be placed in an area where only PCR products are going to be handled. The *ISO 20838:2006* document defines the general requirements for the specific amplification of target nucleic acid sequences and describes the way of detection and confirmation of the amplified nucleic acid sequence. This ISO standard helps the food analytical laboratories for getting comparable and reproducible results. This standard concerns not only to the detection of pathogenic microorganisms from food and feed origin, but of pathogens from environmental samples or to the detection of other investigated microorganisms.

Gloves and laboratory coats should be worn at all times and removed before leaving the room to control amplicon contamination of other locations. All equipment used for amplification and product detection should be dedicated to this room,including adjustable pipettes with plugged, aerosol-barrier tips. This room should be kept under negative pressure.

4.2. PCR LABORATORY EQUIPMENT

Establishment of a PCR-laboratory should meet modern requirements to organization of PCR-analysis with a selected format of detection of results (according to WHO "Establishment of PCR laboratory in developing countries", 2011, and ISO/TS 20836:2005).

To ensure that pre-PCR and post-PCR events remain separated, each room must have its own separate set of equipment, reagents, pipette tips and racks, etc. used in that location only.

The most important devices and equipments for molecular biological work in a food analyzing laboratory:

Sample preparation
- homogenizer
- vortex
- refrigerator
- thermostats
- gloves
- laboratory coat

Nucleic acid extraction
- positive displacement pipettes or pipettors with aerosol-resistant tips
- refrigerator
- freezer
- water bath / dry heat block
- laminar flow biosafety cabinet
- micro-centrifuge
- vortex
- equipment for determination of nucleic acid concentration
- equipment for preparation of Milli-Q water
- gloves
- laboratory coat

Preparation of the reaction mixture
- positive displacement pipettes or pipettors with aerosol-resistant tips dedicated to this area
- micropipettes dedicated to this area
- PCR cabinet (with UV sterilisation)
- freezer
- gloves
- laboratory coats dedicated to this area

Addition of the nucleic acid and PCR amplification
- positive displacement pipettes or pipettors with aerosol-resistant tips dedicated to this area
- dead air box for addition of nucleic acid
- micro-centrifuge
- freezer
- thermal cycler(s) (normal, gradient)
- real-time PCR instrument
- gloves
- laboratory coats

Detection and confirmation of PCR products
- gel electrophoresis equipment
- gel imaging system
- PC with network connection
- hybridisation oven
- incubator
- refrigerator
- freezer
- gloves
- laboratory coats

Thermal cyclers are essential to all PCR methods, and great care should be taken to ensure that they are well-maintained and reliable. The thermal unit temperature of thermal cycler should be tested at least twice a year by the laboratory or under a maintenance agreement to ensure uniform heating throughout the unit. Thermal unit temperature should be tested with an external probe that has been calibrated against a temperature standard. For testing, the probe is placed in several of the wells in the periphery and centre of the instrument. All temperatures should be within the manufacturers' specifications. The amplification programme used in each run should be printed to further verify the conditions of the PCR.

Real-time PCR instruments are equipped to perform fluorescence excitation and detection to monitor amplification throughout the PCR cycles. The design is usually different from the standard thermal cycler, and calibration may be specific to the instrument design. Temperature, light source performance, alignment, and safety devices should be checked and optical systems calibrated.

The machine should be serviced annually. Real time machines should be used with Uninterrupted Power supply (UPS) as these equipment are very delicate, sensitive and also to protect the laser from damage.

Separate centrifuges, including microfuges, are required for pre- and post-PCR procedures. The manufacturers' instructions for calibration should be followed. The centrifuge should be balanced before use to increase bearing life and minimize vibrations.

The vortex is an important equipment required for reagent preparation in the PCR clean room and for nucleic acid extraction.

Automatic, fixed-volume, adjustable, positive-displacement pipettes, and/or micropipettes are used in the PCR laboratory. These should be calibrated quarterly by the manufacturer or a technician. Each pipette should be sterilized according to manufacturers' recommendation on a regular basis or whenever contamination is suspected.

Laboratory users should pay careful attention to the specifications of the hood or cabinet to ensure that it is appropriate for its designated use by the laboratory. Class I cabinets have inward air flow and HEPA-filtered exhaust that provides personal and environmental protection, but no product protection.

Class II and III BSCs filter both air intake and exhaust, and prevent contaminants from entering and leaving the hood (reducing the likelihood of sample and work area contamination). Before use, hoods should be decontaminated using UV light for at least half an hour and cleaned with bleach or other effective nucleic acid inactivating agent. The airflow and HEPA filtration in all hoods should be monitored and certified as per manufacturers' recommendations at least annually.

Separate *refrigerators* for temporary storage of sample, extracted RNA/nucleic acid and final amplification products should be maintained in the respective laboratory. Usually long term storage is not recommended but if needed separate deep freezers (–80 °C) can be maintained.

PCR clean reagents, enzymes, buffer, dNTPS and primers are required to be stored at –20 °C. The primers, dNTPS and water should be stored in small aliquots to avoid freezing and thawing effect and also to rule out contamination issues. To verify that equipment is functioning properly, the laboratory should have a schedule for maintaining equipment. The schedule should include the set-up, calibration, repair, record-keeping, and normal operation of all equipment used in sample analysis. The results of all tests should be documented in an equipment logbook and/or electronic database. The logbook or database should be checked monthly by Quality Control (QC) personnel or the laboratory superviser, and any problems and corrective actions managed. Equipment should be dedicated to a specific laboratory room, and instrument manualsfrom the manufacturer should be available.

Special *tips for PCR analysis* include barrier tips and aerosol-resistant tips, both of which minimize cross-contamination of samples during pipetting. These tips can be purchased pre-sterilized and pre-loaded in hinged racks to provide tip protection and easy access. Pipette tips for PCR analyses should be RNase-free, DNase-free, and pyrogen-free.

Polypropylene tubes that are certified DNase-, RNase-, and pyrogen-free are best recommended for PCR laboratories. The size and style of PCR tubesor reaction plates should be compatible with the block and lid height of the thermocycler/real-time machine. Thin-walled tubes provide the best heat transfer, ensuring that the reaction volume reaches its specified temperature in the shortest amount of time, thereby improving specificity and reproducibility. Tubes containing stored samples and reagents should be centrifuged briefly before opening to ensure that all liquids are at the bottom of the tubes.

"DNA-Technology" offers several variants of establishment of a PCR-laboratory depending on the implemented format of detection of analysis results.

4.2.1. PCR LABORATORY EQUIPMENT FOR REAL-TIME PCR DETECTION (WITH IMPLEMENTATION OF THE DT SERIES THERMAL CYCLERS)

1. Sample preparation area

№	NAME	QUANTITY
1	Lami-type cabinet of II protection type	1
2	Solid State Thermostat "Gnom"	1
3	Centrifuge «Mini Spin» (Eppendorf with a rotor for 12 tubes)	1
4	Centrifuge (Vortex) Microspin FV 2400	1
5	Pipettes (Biohit, Proline Plus series) (0,5-10) mcl –1 pc., (2-20) mcl –1 pc., (20-200) mcl –1 pc., (100-1000) mcl –1 pc.	4
6	Stand "carrousel" for six pipettes (Biohit)	1
7	Stand workstation 200 x 0,2 ml	1
8	Stand workstation 50 x 1,5 ml	1
9	Racks for storing tubes 100 x 1,5 ml	2
10	Pump with flask-trap	1

2. Area for reaction mixture preparation

№	NAME	QUANTITY
1	Lami-type PCR-cabinet	1
2	Centrifuge (Vortex) Microspin FV 2400	1
3	Pipettes (Biohit, Proline Plus series) (0,5-10) mcl –1 pc., (2-20) mcl –1 pc., (20-200) mcl –1 pc., (100-1000) mcl –1 pc.	4
4	Stand "carrousel" for six pipettes (Biohit)	1
5	Stand workstation 200 x 0,2 ml	1
6	Racks for storing tubes 100 x 1,5 ml	2

3. Separate

№	NAME	QUANTITY
1	Detecting amplifier DT*prime* / DT*lite* (*Real-time*) with PC	1

Total number of laboratory equipment pieces

№	NAME	QUANTITY
1	Lami-type PCR-cabinet	1
2	Solid State Thermostat "Gnom"	1
3	Detecting amplifier DT*prime*/DT*lite*(Real-time) with PC	1
4	Pump with flask-trap	1
5	Stand workstation 200 x 0,2 ml	2
6	Stand workstation 50 x 1,5 ml	1
7	Lami-type cabinet of II protection type	1
8	Centrifuge "Pico-17" (with a rotor for 24 tubes)	1
9	Centrifuge (Vortex) Microspin FV 2400	2
10	Pipettes (Biohit, Proline Plus series) (0,5-10) mcl –1 pc., (2-20) mcl –1 pc., (20-200) mcl –1 pc., (100-1000) mcl –1 pc.	8
11	Stand "carrousel" for six pipettes (Biohit)	2
12	Racks for storing tubes 100 x 1,5 ml	4

4.2.2. PCR LABORATORY EQUIPMENT FOR FOR FLASH DETECTION (WITH IMPLEMENTATION OF THE "GENE" / "GENE-4" FLUORESCENCE READERS)

1. Sample preparation area

№	NAME	QUANTITY
1	Lami-type cabinet of II protection type	1
2	Solid State Thermostat "Gnom"	1
3	Centrifuge "Pico-17" (with a rotor for 24 tubes)	1
4	Centrifuge (Vortex) Microspin FV 2400	1
5	Pipettes (Biohit, Proline Plus series) (0,5-10) mcl –1 pc., (2-20) mcl –1 pc., (20-200) mcl –1 pc., (100-1000) mcl –1 pc.	4
6	Stand "carrousel" for six pipettes (Biohit)	1
7	Stand workstation 200 x 0,5 ml	1

8	Stand workstation 50 x 1,5 ml	1
9	Racks for storing tubes 100 x 1,5 ml	2
10	Pump with flask-trap	1

2. Area for reaction mixture preparation

№	NAME	QUANTITY
1	Lami-type PCR-cabinet	1
2	Centrifuge (Vortex) Microspin FV 2400	1
3	Pipettes (Biohit, Proline Plus series) (0,5-10) mcl – 1 pc., (2-20) mcl – 1 pc., (20-200) mcl – 1 pc., (100-1000) mcl – 1 pc.	4
4	Stand "carrousel" for six pipettes (Biohit)	1
5	Stand workstation 200 x 0,5 ml	1
6	Racks for storing tubes 100 x 1,5 ml	2

3. Separate

№	NAME	QUANTITY
1	Multichannel amplifier Tercyc	1
2	PCR-detector "Gene"/"Gene-4"	1
3	Stand workstation "Gene+"	1
4	Laptop with pre-installed specialized software	1

Total number of laboratory equipment pieces

№	NAME	QUANTITY
1	Lami-type PCR-cabinet	1
2	Solid State Thermostat "Gnom"	1
3	Multichannel amplifier Tercyc	1
4	PCR-detector "Gene"/"Gene-4"	1
5	Stand workstation "Gene+"	1
6	Laptop with pre-installed specialized software	1
7	Pump with flask-trap	1
9	Stand workstation 200 x 0,5 ml	2
10	Stand workstation 50 x 1,5 ml	1
11	Lami-type cabinet of II protection type	1
12	Centrifuge "Pico-17" (with a rotor for 24 tubes)	1

13	Centrifuge (Vortex) Microspin FV 2400	2
14	Pipettes (Biohit, Proline Plus series) (0,5-10) mcl – 1 pc., (2-20) mcl – 1 pc., (20-200) mcl – 1 pc., (100-1000) mcl – 1 pc.	8
15	Stand "carrousel" for six pipettes (Biohit)	2
16	Racks for storing tubes 100 x 1,5 ml	4

4.2.3. PCR LABORATORY EQUIPMENT FOR ELECTROPHORETIC DETECTION

1. Sample preparation area

№	NAME	QUANTITY
1	Lami-type cabinet of II protection type	1
2	Solid State Thermostat "Gnom"	1
3	Centrifuge "Pico-17" (with a rotor for 24 tubes)	1
4	Centrifuge (Vortex) Microspin FV 2400	1
5	Pipettes (Biohit, Proline Plus series) (0,5-10) mcl – 1 pc., (2-20) mcl – 1 pc., (20-200) mcl – 1 pc., (100-1000) mcl – 1 pc.	4
6	Stand for pipettes	1
7	Stand workstation 200 x 0,5 ml	1
8	Stand workstation 50 x 1,5 ml	1
9	Racks for storing tubes 100 x 1,5 ml	2
10	Pump with flask-trap	1

2. Area for reaction mixture preparation

№	NAME	QUANTITY
1	Lami-type PCR-cabinet	1
2	Centrifuge (Vortex) Microspin FV 2400	1
3	Pipettes (Biohit, Proline Plus series) (0,5-10) mcl – 1 pc., (2-20) mcl – 1 pc., (20-200) mcl – 1 pc., (100-1000) mcl – 1 pc.	4
4	Stand for pipettes	1
5	Stand workstation 200 x 0,5 ml	1
6	Racks for storing tubes 100 x 1,5 ml	2

3. Area for electrophoresis

№	NAME	QUANTITY
1	Multichannel amplifier Tercyc	1
2	Power supply for electrophoresis Elf-4	1
3	Chamber for electrophoresis SE-2	1
4	Transilluminator (Vilber Lourmat) ECX-15M	1
5	Pipette (0,5-10) mcl	1
6	Stand for pipettes	1
7	Video system for recording electrophoresis results (Gel Imager)	1

Total number of laboratory equipment pieces

№	NAME	QUANTITY
1	Lami-type cabinet of II protection type	1
2	Lami-type PCR-cabinet	1
3	Solid State Thermostat "Gnom"	1
4	Multichannel amplifier Tercyc	1
5	Power supply for electrophoresis Elf-4	1
6	Video system for recording electrophoresis results (Gel Imager)	1
7	Pump with flask-trap	1
8	Stand workstation 200 x 0,5 ml	2
9	Stand workstation 50 x 1,5 ml	1
10	Centrifuge "Pico-17" (with a rotor for 24 tubes)	1
11	Centrifuge (Vortex) Microspin FV 2400	2
12	Chamber for electrophoresis SE-2	1
13	Transilluminator (Vilber Lourmat) ECX-15M	1
14	Pipettes (Biohit, Proline Plus series) (0,5-10) mcl –1 pc., (2-20) mcl –1 pc., (20-200) mcl –1 pc., (100-1000) mcl –1 pc.	9
15	Stand for pipettes	3
16	Racks for storing tubes 100 x 1,5 ml	4

4.3. REGULATORY DOCUMENTS

Proper organization of a PCR-laboratory, obtaining permissions for operation and issuance of adequate results of analyses requires compliance with the following regulatory documents:

ISO/IEC 17025:1999 «General requirements for the competence of testing and calibration laboratories»

ISO 22174:2005 «Microbiology of food and animal feeding stuffs – Polymerase chain reaction (PCR) for the detection of food-borne pathogens – General requirements and definitions»

ISO 20837:2006 «Microbiology of food and animal feeding stuffs – Polymerase chain reaction (PCR) for the detection of food-borne pathogens – Requirements for sample preparation for qualitative detection»

ISO 20838:2006 «Microbiology of food and animal feeding stuffs – Polymerase chain reaction (PCR) for the detection of food-borne pathogens – Requirements for amplification and detection for qualitative methods»

The *validation* can be performed by following the relevant ISO, CEN (European Committee for Standardization), AOACI (Association of Analytical Communities International) or AFNOR (Association Francoise de Normalization) standard procedures.

The internationally recognized protocol to which proficiency testing schemes should comply is the IUPAC/AOAC/ISO Harmonized Protocol.

5. ADVANTAGES OF THE PCR-METHOD

PCR-method is one of the most quickly developing fields in molecular genetic analysis, which is used in practice of diagnostic laboratories. Wide implementation of the given method made it possible to significantly increase the quality of laboratory analysis, to reduce the analysis time and to automate the system of processing, storing and obtaining the results.

Possibilities of a present-day PCR-laboratory enable conducting multifactorial, multiplex, quantitative analyses, detecting nucleic acids of various microorganisms in the obtained samples.

In diagnostics of infectious diseases real-time PCR provides a possibility to detect the presence of an infectious agent (qualitative analysis) and concentration of DNA/cDNA in the sample (quantitative analysis).

Qualitative analysis is used (and is sufficient) for detecting unconditional pathogens, such as Gonococci, Chlamydia, Trichomonads. At present there are no protocols for treatment of these infections which would take into account the quantity of the infectious agent.

Quite another situation is for conditional pathogens – qualitative analysis for this group of microorganisms is insufficient. As it follows from the name "conditional", their presence does not mean presence of a disorder and it is important not to detect presence of the microorganism in the analyzed sample, but to evaluate its concentration as well. In this case it is reasonable to use quantitative analysis with real-time detection of results. Besides, quantitative analysis is recommended for determining the viral load and strategy of treatment for viral hepatitis B and C, HIV.

It should be noted that in a number cases there arises a possibility of establishing the diagnosis within a short time period and a high degree of reliability for exclusion of development of complications. Implementation of traditional methods – the cultural and the microscopic one – does not make it possible to realize the given possibility due to objective reasons.

For example, main tasks of microscopy are:

- detecting infectious agents in the clinical material;
- investigatory identification on the basis of determining characteristic morphological and tinctorial features of microorganisms;
- investigating stained smear of colonies of pure cultures.

This method is considered to be the quickest and cheapest, its implementation is connected with minimal requirements to laboratory organizations.

Nevertheless, there are a number of restrictions in the use of microscopy for diagnosing infectious diseases:

- low sensitivity of the method;
- subjectivity of assessment of results;
- limited range of detected morhotypes;
- approximate quantity analysis.

The culture method, together with microscopy of organisms, is included in to the "golden standard" of diagnostics and makes it possible:

- to detect all live cultured microorganisms;
- to determine the antibiotic resistance of the detected microorganisms.

Nevertheless, the culture method has objective restrictions:

- long periods of culturing – from five days to two months;
- increased requirements to transporting and storing biomaterial;
- absence of possibility of culturing most anaerobic microorganisms;
- increased requirements to laboratory organization and diagnostic environments.

Introduction into the laboratory practice and wide use of molecular biological analysis methods, first of all, PCR, provides a number of advantages for modern laboratories:

- **high speed of analysis** – even the most complex multiparameter methods take less than a day to the moment of collecting the material for analysis and providing results of the analysis;
- **detecting concentration of microorganisms in biological material;**
- **high repeatability of results;**
- **maximal sensitivity and specificity;**
- **standardized technological process;**
- possibility of conduct diagnostics of a whole number of infectious agents from one sample of the biological material (in case of use of other methods there can arise a need of collecting several samples of the biomaterial of one patient, which can be traumatic and therefore undesirable);
- obtaining results irrespective of the stage of the infectious process;
- conducting analysis irrespective of the prescription for the patient of antibiotic therapy;
- determining genetic factors underlying the antibiotic resistance of bacteria (possibility of choosing the most efficient therapeutic scheme).

An important aspect of PCR-diagnostics is a possibility of conducting genetic analysis, including determining the risk of somatical pathologies development and drug tolerability (e.g. warfarin resistance/sensitivity test).

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