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**Federal State Autonomous Educational Institution
Higher Education "Peoples' Friendship University of Russia"**

Medical Institute

(name of the main training unit (PMO)-developer of the EP HE)

WORK PROGRAM OF THE DISCIPLINE

Molecular methods for diagnosing phytopathogens

(name of discipline/module)

Recommended by ISSS for the direction of training/specialty:

35.04.04. Agronomy

(code and name of the direction of training/specialty)

The development of the discipline is carried out within the framework of the implementation of the main professional educational program of higher education (EP HE):

Agronomy

(name (profile/specialization) ep he)

1. THE PURPOSE OF MASTERING THE DISCIPLINE

The purpose of mastering the discipline "Molecular methods for diagnosing phytopathogens" is to obtain basic knowledge about the methods and ways of spreading a viral infection, measures to prevent plant infection and methods of localization of lesions, familiarization with modern methods of identification and diagnosis of viruses.

2. REQUIREMENTS FOR THE RESULTS OF MASTERING THE DISCIPLINE

Mastering the discipline "Molecular methods for diagnosing phytopathogens" is aimed at the formation of the following competencies in students: OPK-1.2; OPK-4.2; OPK-4.3; PC-4.5; PC-4.6; PC-7.1; PC-7.2

OPK-1.1; OPK-1.2; OPK-4.2; OPK-4.3; PC-2.1; PC-5.1; PC-7.1; PC-7.2

Table 2.1. List of competencies formed in students during the development of the discipline (results of mastering the discipline)

Code	Competence	Competency Achievement Indicators (within the framework of this discipline)
OPK-1.1	Demonstrates knowledge of the main methods of analysis of the achievements of science and production in agronomy	OPK-1.1.1 Able to apply in practice knowledge about the scheme of identification of phytopathogen by molecular methods
OPK – 1.2	Uses methods of solving problems in the development of agronomy based on the search and analysis of modern achievements of science and production	OPK-1.2.1 Uses in professional activities ideas about the basics of the molecular structure of DNA and RNA molecules, their biological and physicochemical properties OPK-1.2.2 Uses in professional activities methods for diagnosing phytopathogens, including the ELISA method and PCR modifications
OPK – 4.2	Uses information resources, scientific, experimental and instrumental base for research in agronomy	OPK-4.2.1 Uses modern equipment in the laboratory for conducting tests by PFR and ELISA methods OPK-4.2.2 Uses skills in working with analytical samples of the material for the isolation of DNA / RNA, its amplification and detection of phytopathogens
OPK – 4.3	Formulates the results obtained in the course of solving research problems	OPK-4.3.1 Able to interpret the results of modern molecular genetic diagnostic methods, including bioinformatic analysis of nucleotide sequences
PC-2.1	Develops methods of conducting experiments	PC – 2.1.1 Participates in the development of regulatory documents for the diagnosis of pests PC – 2.1.2 Participates in the testing and development of new tests for species

		diagnostics of pathogens
PC-5.1	Draws up a research program to study the effectiveness of agricultural techniques	PP-5.1.1 Introduces rapid diagnostic methods in the process of establishing the phytosanitary state of fields and gardens to develop a program to combat identified phytopathogens
PC – 7.1	Recognizes quarantine objects and identifies quarantine pests and pathogens	PC – 7.1.1 Owns methods of species identification of fungi, bacteria, nematodes, viruses, viroids and phytoplasmas related to quarantine and closely related
PC – 7.2	Conducts examination of crops and crop products for the presence of quarantine facilities	SC – 7.2.1 Owns methods and techniques for conducting phytosanitary studies of plant material to search for quarantine phytopathogen species, including sampling and preparation of analytical samples.

3. MESTO DISCIPLINE IN THE STRUCTURE OF THE OP VO

The discipline "**Molecular methods for the diagnosis of phytopathogens**" refers to *the elective* part of the block B1.B.DV.03.01 OP VO.

Within the framework of the OP HE, students also master other disciplines and / or practices that contribute to the achievement of the planned results of mastering the discipline "**Molecular methods for diagnosing phytopathogens**".

Table 3.1. List of components of the OP HE that contribute to the achievement of the planned results of the discipline

Code	Competence	Previous disciplines/modules, practices*	Subsequent disciplines/modules, practices*
OPK-1.1	Demonstrates knowledge of the main methods of analysis of the achievements of science and production in agronomy	Phytopathology Biological method of plant protection Work with scientific literature Fundamentals of Scientific Communication Plant protection in organic farming Prognosis of pests and diseases Phytosanitary risk analysis	Instrumental research methods Instrumental research methods Plant quarantine Biotechnology in plant protection
OPK – 1.2	Uses methods of solving problems in the development of agronomy based on the search and analysis of modern achievements of science and production	Phytopathology Biological method of plant protection Work with scientific literature Fundamentals of Scientific Communication Plant protection in organic farming	Instrumental research methods Instrumental research methods Plant quarantine Biotechnology in plant protection

		Prognosis of pests and diseases Phytosanitary risk analysis	
OPK – 4.2	Uses information resources, scientific, experimental and instrumental base for research in agronomy	Phytopathology Biological method of plant protection Work with scientific literature Fundamentals of Scientific Communication Plant protection in organic farming Prognosis of pests and diseases Phytosanitary risk analysis	Instrumental research methods Instrumental research methods Plant quarantine Biotechnology in plant protection
OPK – 4.3	Formulates the results obtained in the course of solving research problems	Phytopathology Biological method of plant protection Work with scientific literature Fundamentals of Scientific Communication Plant protection in organic farming Prognosis of pests and diseases Phytosanitary risk analysis	Instrumental research methods Instrumental research methods Plant quarantine Biotechnology in plant protection
PC-2.1	Develops methods of conducting experiments	Phytopathology Biological method of plant protection Work with scientific literature Fundamentals of Scientific Communication Plant protection in organic farming Prognosis of pests and diseases Phytosanitary risk analysis	Instrumental research methods Instrumental research methods Plant quarantine Biotechnology in plant protection
PC-5.1	Draws up a research program to study the effectiveness of agricultural techniques	Phytopathology Biological method of plant protection Work with scientific literature Fundamentals of Scientific Communication Plant protection in organic farming	Instrumental research methods Instrumental research methods Plant quarantine Biotechnology in plant protection

		Prognosis of pests and diseases Phytosanitary risk analysis	
PC – 7.1	Recognizes quarantine objects and identifies quarantine pests and pathogens	Phytopathology Biological method of plant protection Work with scientific literature Fundamentals of Scientific Communication Plant protection in organic farming Prognosis of pests and diseases Phytosanitary risk analysis	Instrumental research methods Instrumental research methods Plant quarantine Biotechnology in plant protection
PC – 7.2	Conducts examination of crops and crop products for the presence of quarantine facilities	Phytopathology Biological method of plant protection Work with scientific literature Fundamentals of Scientific Communication Plant protection in organic farming Prognosis of pests and diseases Phytosanitary risk analysis	Instrumental research methods Instrumental research methods Plant quarantine Biotechnology in plant protection

* - is filled in accordance with the competence matrix and the SPMS OP VO

4. SCOPE OF DISCIPLINE AND TYPES OF EDUCATIONAL WORK

The total labor intensity of the discipline "**Molecular methods for diagnosing phytopathogens**" is 4 credits.

Table 4.1. Types of educational work by periods of mastering the EP HE for **full-time** education

Type of educational work		TOTAL, aca.hrs.	Semester(s)			
			4	5		
<i>Contact work, ac.ch.</i>		34	34	34		
Including:						
Lectures (LC)						
Laboratory works (LR)		34	14	20		
Practical/Seminar Classes (FPs)						
<i>Independent work of students, ac.ch.</i>		59	30	22		
<i>Control (exam /test with grade), ac.ch.</i>		15	7	8		
Overall labor intensity of the discipline	aca.hrs.	108	51	57		
	Hrs.ed.	3	1	2		

Table 4. 2. Types of educational work by periods of mastering the OP HE for **full-time and part-time** education

Type of educational work		TOTAL, aca.hrs.	Semester(s)			
			1	2		
<i>Contact work, ac.ch.</i>		26	26			
Including:						
Lectures (LC)						
Laboratory works (LR)		26	26			
Practical/Seminar Classes (FPs)						
<i>Independent work of students, ac.ch.</i>		57	57			
<i>Control (exam /test with grade), ac.ch.</i>		25	25			
Overall labor intensity of the discipline	aca.hrs.	108	108			
	Hrs.ed.	3	3			

Table 4. 3. Types of educational work by periods of mastering the OP HE for **part-time** education

Type of educational work		TOTAL, aca.hrs.	Semester(s)			
			Winters.	Years.		
<i>Contact work, ac.ch.</i>		10	10			
Including:						
Lectures (LC)						
Laboratory works (LR)		10	10			
Practical/Seminar Classes (FPs)						
<i>Independent work of students, ac.ch.</i>		89	89			
<i>Control (exam /test with grade), ac.ch.</i>		9	9			
Overall labor intensity of the discipline	aca.hrs.	108	108			
	Hrs.ed.	3	3			

5. CONTENT OF THE DISCIPLINE

Table 5.1. The content of the discipline (module) by types of educational work

Name of the discipline section	Contents	Type of educational work*
Section 1 Introduction to Molecular Biology	Topic 1.1. The subject and history of molecular biology in the context of diagnostics. The structure of DNA and its properties. ELISA: the principle of the method and comparison with PCR.	LR, WED
Section 2 The main stages and sections of molecular genetic diagnostic methods	Topic 2. 1. Basics of PCR methods. Classical PCR	LR, WED
	Topic 2. 2. Electrophoresis method for visualization of PCR results	LR, WED
	Topic 2. 3. Real-time PCR - qualitative and quantitative analysis	LR, WED

	Topic 2. 4. Modifications of the PCR method. Nested, ISSR, RFPL, LAMP, Drop-digital.	LR, WED
	Topic 2. 5. Interpretation of PCR results. Schemes of analysis. Practical application.	LR, WED
Section 3 Analysis of nucleotide sequences	Topic 3. 1. Sequencing method: Principle, steps.	LR, WED
	Topic 3. 2. Sequencing Method. Interpretation of Results. Bioinformational Analysis and Application in Practice.	LR, WED
	Topic 3. 3. Phylogenetic analysis	LR, WED
Section 4 Genetically engineered organisms.	Topic 4. 1. Fundamentals of Genetic Engineering in Agriculture: The Use of Developments and Their Impact on the Environment	LR, WED
	Topic 4. 2. Methods of detection and diagnosis of genetically modified plants. International legislative practice of GMO control.	LR, WED
Section 5 Cloning method in the diagnosis of phytopathogens.	Topic 5.1. Molecular DNA cloning	LR, WED
	Topic 5.2. Stages of formation of diagnostic protocols for species diagnostics of phytopathogens	LR, WED
	Topic 5.3. Scientific and practical significance of the use of DNA and RNA in the effective diagnosis of phytopathogens and pests of agricultural crops	LR, WED

* - is filled only in **full-time** forms of training: *LC* - lectures; *LR* - laboratory work; *SZ* - seminar classes.

6. MATERIAL AND TECHNICAL SUPPORT OF DISCIPLINE

Table 6.1. Logistics of discipline

Audience type	Equipping the classroom	Specialized educational/laboratory equipment, software and materials for mastering the discipline (if necessary)
Specialized audience	An auditorium for practical work, individual consultations, current control and intermediate certification, equipped with a set of specialized furniture and equipment. (audiences 310, 238)	Comof specialized furniture Mobile Projector
Educational and	Laboratory of Molecular	Amplifier for classical PCR

Audience type	Equipping the classroom	Specialized educational/laboratory equipment, software and materials for mastering the discipline (if necessary)
Scientific Laboratory	Genetic Diagnostic Methods (235, 439)	Set of dispensers Solid-state thermostat Vortex Centrifuge
For independent work of students	Auditorium for independent work of students (can be used for lectures and consultations), equipped with a set of specialized furniture (room 310)	Set of specialized furniture Mobile Projector

* - the audience for independent work of students is indicated **NECESSARILY!**

7. EDUCATIONAL, METHODOLOGICAL AND INFORMATION SUPPORT OF THE DISCIPLINE

Main literature:

Publications:

1. D.V. Rebrikov. Real-time PCR. Ed. "Laboratory of Knowledge", 2015
2. V.V.Lukashov. Molecular evolution and phylogenetic analysis. Ed. "Binomial", 2009
3. D. V. Rebrikov, V. V. Ilyinsky, D. O. Korostin, E. S. Shubina. NGS High Performance Sequencing

Further reading:

Electronic and printed full-text materials:

1. "Molecular Biology (Structure and Biosynthesis of Nucleic Acids)", "Graduate School", 1990.
2. Lewin B. "Genes", Publishing House "The World", 1987
3. Mamontov S.G., Zakharov V.B. Obschchaya biologiya. M.; Ed. "Higher School", 1996

Resources of the information and telecommunication network "Internet":

1. RUDN University EBS and third-party EBS, to which university students have access on the basis of concluded contracts:

- Electronic library system RUDN University – EBS RUDN university
<http://lib.rudn.ru/MegaPro/Web>
- EBS "University Library Online" <http://www.biblioclub.ru>

2. Databases and search engines:

- NCBI: <https://p.360pubmed.com/pubmed/>
- RUDN University Bulletin: access mode from the territory of RUDN University and remotely
<http://journals.rudn.ru/>
- Scientific Library Elibrary.ru: access by IP-addresses of RUDN University at the address:
<http://www.elibrary.ru/defaultx.asp>
 - Electronic resource: EPPO global database URL <https://gd.eppo.int/>
 - Electronic resource: Classical and molecular biology URL <http://molbiol.ru/>

Educational and methodical materials for independent work of students when mastering the discipline / module*:

1. Methodical instructions for students on mastering the discipline "PCR" of the company "DNA-Technology"
 2. Stepik training application for advanced training and independent work of students
- * - all educational and methodological materials for independent work of students are placed in accordance with the current procedure on the page of **the discipline in TUIS!**

8. EVALUATION MATERIALS AND POINT-RATING SYSTEM FOR ASSESSING THE LEVEL OF FORMATION OF COMPETENCIES IN THE DISCIPLINE

Evaluation materials and a point-rating system* for assessing the level of formation of competencies (part of competencies) based on the results of mastering the discipline "**Molecular methods for diagnosing phytopathogens**" are presented in the Appendix to this Work Program of the discipline.

* - OM and BRS are formed on the basis of the requirements of the relevant local regulatory act of RUDN University.

DEVELOPERS:

Senior Lecturer at the
Agrobiotechnology Department

Position, BCD

Signature

Bondarenko G.N.

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HEAD OF BCD:

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Federal State Autonomous Educational Institution
Higher education
"PEOPLES' FRIENDSHIP UNIVERSITY OF RUSSIA"
Agrarian-Technological Institute

VALUATION FUND

BY DISCIPLINE

Molecular methods for diagnosing phytopathogens

Direction of training

35.04.04 AGRONOMY

2022

Evaluation criteria:

(in accordance with the current regulatory framework)

Compliance of grading systems (previously used grades of final academic performance, ECTS grades and the point-rating system (BRS) of assessments of current academic performance).

BRS Scores	Traditional Assessments of the Russian Federation	Evaluation ECTS
95 - 100	5	A
86 - 94		B
69 - 85	4	C
61 - 68	3	D
51 - 60		E
31 - 50	2	FX
0 - 30		F
51-100	Credit	Passed

Explanation of the rating table:

Description of ECTS ratings

A	"Excellent" - the theoretical content of the course is mastered completely, without gaps, the necessary practical skills of working with the mastered material are formed, all the educational tasks provided for by the training program are completed, the quality of their implementation is estimated by the number of points close to the maximum.
B	"Very good" - the theoretical content of the course is mastered completely, without gaps, the necessary practical skills of working with the mastered material are mainly formed, all the educational tasks provided for by the training program are completed, the quality of most of them is estimated by the number of points close to the maximum.
C	"Good" - the theoretical content of the course is mastered completely, without gaps, some practical skills of working with the mastered material are not sufficiently formed, all the educational tasks provided for by the training program are fulfilled, the quality of none of them is assessed by a minimum number of points, some types of tasks are performed with errors.

D	"Satisfactory" - the theoretical content of the course is partially mastered, but the gaps are not significant, the necessary practical skills of working with the mastered material are mainly formed, most of the educational tasks provided for by the training program have been completed, some of the completed tasks may contain errors.
E	"Mediocre" - the theoretical content of the course is partially mastered, some practical skills are not formed, many of the training tasks provided for by the training program have not been completed, or the quality of some of them is estimated by the number of points close to the minimum.
FX	"Conditionally unsatisfactory" - the theoretical content of the course is partially mastered, the necessary practical skills of work are not formed, most of the training tasks provided for by the training program have not been completed, or the quality of their implementation is estimated by a number of points close to the minimum; with additional independent work on the course material it is possible to improve the quality of educational tasks.
F	"Certainly unsatisfactory" - the theoretical content of the course has not been mastered, the necessary practical skills of work have not been formed, the all-completed training tasks contain gross errors, additional independent work on the course material will not lead to any significant improvement in the quality of the performance of educational tasks.

Positive grades, in which the course is counted as completed by the student, are grades A, B, C, D and E.

A student who has received an **FX** grade in the discipline of the educational program is obliged, after consultation with the appropriate teacher, to successfully complete the required minimum amount of training work provided for in the training program within the time limits established by the training part, and to submit the results of these works to this teacher. If the quality of the work is considered satisfactory, the final FX score is increased to E and the trainee is allowed for further training.

In the event that the quality of the training work remains unsatisfactory, the final grade is reduced to F and the trainee is submitted for expulsion. In the event of receiving an F or FX grade, the trainee is submitted for deduction regardless of whether he has any other debts in other disciplines.

(Order of the Rector of RUDN University No. 996 of 27.12.2006)

Evaluation criteria:

(in accordance with the current regulatory framework)

№ p/n	Indicators / Evaluation Criteria	<i>It's cool</i>	<i>Ok</i>	<i>satisfactorily</i>	<i>unsatisfactorily</i>
1.	Completeness of the reflection of the necessary information in each question	Fully	Sufficiently	Partly	Not available
2.	Having the student's own comments in those sections where necessary.	Fully	Sufficiently	Partly	Missing
3.	Completeness and validity of the conclusion and conclusions	Fully substantiated	Sufficiently substantiated	Insufficiently substantiated	Not justified

Note:

1. An "excellent" grade is given if all the criteria are "excellent" and no more than one "good" criterion.
2. A rating of "good" is given if all the criteria are "good" and "excellent", no more than one criterion "satisfactory".
3. A rating of "satisfactory" is given if all evaluation criteria are positive, no more than one criterion is "unsatisfactory".
4. A rating of "unsatisfactory" is obtained against the criteria of more than one unsatisfactory rating.
- 5.

Number of points	Final score
<5	Unsatisfactorily
5-10	Satisfactorily
10-15	Ok
15-20	Excellent

Tests in the discipline "Molecular methods of diagnosis of phytopathogens" (example)

1. T-plasmids are:
 - (a) Small DNA molecules physically separated from chromosomes and capable of autonomous replication+
 - b) molecules composed of DNA fragments of different origins
 - c) freely existing molecules that help amplify DNA fragments that are located between two sufficiently closely spaced inverted microsatellites
 - d) there is no correct answer
2. Phylogenetic analysis is:
 - a) graphical representation of the analysis of the similarity of nucleotide sequences of different types+
 - b) mathematical analysis of the similarities and differences of bacteria by apomorphies
 - c) construction of dendrograms for certain groups of organisms when studying their classification
 - d) all answers are correct
3. Sequencing is:
 - (a) Nucleotide sequence preparation process+
 - b) the process of embedding a foreign gene into a T-plasmid during cloning
 - c) the process of lengthening the DNA chain during amplification
 - d) there is no correct answer
4. A primer is:
 - (a) A fragment of a T-plasmid where restriction occurs for gene insertion
 - b) the terminator with which the chain breaks during sequencing
 - c) a synthetic oligonucleotide used as a seed for DNA replication+
 - d) there is no correct answer
5. Application of the sequencing method:
 - (a) Analysis of restriction fragment lengths

- b) species identification of phytopathogens
 - c) phylogenetic analysis of groups of closely related organisms
 - d) all answers are correct+
6. The principle of the "nearest neighbors" method:
- (a) Calculation of pairwise differences between the respective genes of all species involved in the analysis, with the consequence that the more differences are found, the greater the "distance" between the species will be+
 - b) the species, one by one, undergo a tray analysis procedure until a single, fully permitted tree is found and all identical species are combined into hoards.
 - c) the factor is taken into account - the posteriori probability, which is calculated on the basis of both the initial data and the results of the analysis obtained
 - d) there is no correct answer
7. Clades are:
- (a) Graphical representation of the relationship between taxa
 - b) a group of two or more taxa, including a common precursor and all monophyletic groups derived from it+
 - c) the place of connection of branches, uniting representatives of one taxonomic group (one species, similar strains)
 - d) there is no correct answer
8. **A couple** of logs are:
- (a) Homologous genes derived from a common progenitor gene during natural speciation
 - b) homologous genes resulting from horizontal gene transfer between organisms
 - c) there is no correct answer+ (doubling?)
9. Restrictions are:
- (a) An enzyme that catalyzes the compound of two molecules to form a new chemical bond
 - b) nuclease enzymes that catalyze RNA degradation
 - c) a group of enzymes belonging to the class of hydrolases that catalyze the reaction of hydrolysis of nucleic acids +
 - d) there is no correct answer
10. Contamination is:
- (a) Reinfection of samples (samples) with biological material among themselves
 - b) a positive result of negative controls when setting UP PCR
 - c) the entry of foreign DNA / RNA into the sample during the release of nucleic acids
 - d) all answers are correct+
11. Positive PCR control is used to:
- (a) Clean zone verification
 - b) verification of the nucleic acid isolation process
 - c) checking the amplification in the device+
 - d) all answers are correct
12. Recombinant DNA is:
- a) a DNA molecule that is obtained by reverse transcription
 - b) genetic material of natural origin that participates in cloning
 - c) a molecule composed of DNA fragments of different origins+
 - d) there is no correct answer

13. A transgenic plant is:
- (a) A plant resulting from genome editing
 - b) a plant whose genetic makeup has been artificially altered+
 - c) a plant that has received a new trait as a result of breeding activities
 - d) there is no correct answer
14. A vector is:
- (a) The DNA sequence encoding the production of the necessary protein
 - b) DNA or RNA molecules that are capable of replicating in certain cells and can accept and carry foreign DNA or RNA+
 - c) synthetic oligonucleotide capable of activating the amplification process of recombinant DNA
 - d) there is no correct answer
15. Is it allowed to grow GM plants in Russia?
- (a) Only as animal feed
 - b) Only as a mandatory export of products
 - c) Only within the framework of research activities+
 - d) there is no correct answer
16. In which EU countries is it allowed to grow GM plants?
- a) France, Germany, Czech Republic, Portugal
 - b) Spain, Italy, Slovakia, Macedonia
 - c) Slovakia, Czech Republic, Portugal, Spain+
 - d) there is no correct answer
17. Orthologs are:
- (a) Homologous genes resulting from the doubling of the progenitor gene
 - b) homologous genes derived from a common progenitor gene during natural speciation+
 - c) there is no correct answer
18. Apomorphies are:
- a) features that help to unite different species in a clade
 - b) features reflecting the fundamental difference between species in the clade+
 - c) signs indicating the possibility of gene transfer from cell to cell
 - d) there is no correct answer
19. RFLP is:
- a) the process of amplification of the DNA section using microsatellite primers+
 - b) computer analysis of the obtained nucleotide DNA sequences
 - c) analysis of the lengths of restriction fragments
 - d) there is no correct answer
20. The main actions in the elimination of contamination:
- (a) Use of UV and chlorine-containing solutions+
 - b) use of replaceable gowns and disposable gloves
 - c) use of disposable utensils and non-powdered talcum powder gloves
 - d) there is no correct answer (*all conditions are important*)
21. Analysis process *E. coli* with embedded DNA section:
- a) selection of white colonies on a nutrient medium with antibiotics
 - b) selection of blue colonies on a nutrient medium with antibiotics +

- c) selection of pink colonies on a nutrient medium with antibiotics
- d) there is no correct answer

22. Terminator is:

- a) labeled didisoxynucleotide, which, upon amplification, breaks the chain+
- b) labeled disoxynucleotide, which, when amplified, breaks the bond between the fluorophore and the fluorescence damper
- c) labeled oligonucleotide that lengthens the chain in Seq-PCR
- d) there is no correct answer

23. Application of phylogenetic analysis:

- (a) Comparison of dna of organisms for taxonomic analysis
- b) comparison of DNA of organisms to search for species-specific diagnostic targets
- c) all answers are correct+

24. The presence of which zone in the laboratory is not necessary when using PCR in "real time":

- (a) Nucleic acid release zones
- b) electrophoresis+ zones
- c) areas of preparation of reaction mixtures
- d)there is no correct answer