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SPECIFIC CHARACTERISTICS OF RAPID DIAGNOSIS IN PERIODONTOLOGY

Abstract. Currently, the advanced studies have been justified and implemented in the Republic of Belarus that enable the production and a practical use of a domestic rapid test and allow dentists to apply microbiological diagnosis methods.

The objective of the study was to develop a domestic rapid test for periodontology, indicating the procedure for preparatory clinical and laboratory measures using PCR and trypsin-like activity of periopathogenic microflora (*Treponema denticola, Porphyromonas gingivalis, Bacteroides forsythus*)

Dental examination was performed in 60 patients with clinical signs of generalized periodontitis and a 5–6 mm deep pathological dentogingival pocket (PDGP), aged 35–44 (the main group), and 140 patients without signs of periodontal diseases, aged 20–24 (the control group). These patients were selected among the visitors of Chair No. 3 of the Belarusian State Medical University. The laboratory diagnosis was made using PCR and evaluating the trypsin-like activity of periopathogenic microflora.

The algorithm of preparatory clinical and laboratory measures was suggested for diagnosis of periodontal diseases, including material sampling and identification of periopathogenic microorganisms in the sample using a biochemical method; sampling, preservation and storage of material for quantitative PRC testing; and the identification of the genetic material of periopathogenic microorganisms by means of a real-time quantitative PRC method.

The basic principles of microbiological diagnosis in periodontology enable making a true diagnosis, planning treatment and assessing the treatment results.

We examined the content of the gingival sulcus and/or the periodontal pocket in patients who have periodontal diseases. It was noted that the most rational tools for removing the periodontal pocket content in patients with periodontal diseases was a paper pin.

The development of a domestic express test in periodontology demonstrated the need of preparatory clinical and laboratory activities using PCR and trypsin-like activity of periopathogenic microflora (*Treponema denticola*, *Porphyromonas gingivalis*, *Bacteroides forsythus*).

Keywords: rapid diagnosis, periodontology, clinical and laboratory measures, periodontal tissue

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ОСОБЕННОСТИ ЭКСПРЕСС-ДИАГНОСТИКИ В ПЕРИОДОНТОЛОГИИ

Аннотация. В настоящее время в Республике Беларусь перспективным направлением в периодонтологии являются разработка, производство и внедрение отечественного экспресс-теста, который даст возможность врачу широко использовать методы микробиологической диагностики в стоматологической практике.

Цель исследования – разработать отечественный экспресс-тест в периодонтологии и обозначить порядок подготовительных клинико-лабораторных мероприятий, используя ПЦР и трипсиноподобную активность периопатогенной микрофлоры (*Treponema denticola*, *Porphyromonas gingivalis*, *Bacteroides forsythus*).

С использованием методов клинического обследования тканей периодонта проведено стоматологическое обследование 60 пациентов в возрасте 35–44 лет с клиническими признаками генерализованного периодонтита и глубиной патологического зубодесневого кармана 5–6 мм (основная группа) и 140 пациентов в возрасте 20–24 года без признаков болезней периодонта (контрольная группа), обратившихся на 3-ю кафедру терапевтической стоматологии БГМУ. Лабораторная диагностика заключалась в оценке трипсиноподобной активности периопатогенной микрофлоры с помощью ПЦР.

Предложен алгоритм подготовительных клинико-лабораторных мероприятий в диагностике болезней периодонта, включающий забор материала и выявление в образце периопатогенных микроорганизмов с использованием биохимического метода; забор материала, его консервация и хранение для исследования методом количественной ПЦР; выявление генетического материала периопатогенных микроорганизмов методом количественной ПЦР в режиме реального времени.

Основные принципы микробиологической диагностики в периодонтологии дают возможность достоверно поставить диагноз, планировать лечение и оценивать его результаты.

Изучено содержимое десневой борозды и/или периодонтального кармана у пациентов с болезнями периодонта. Для извлечения содержимого из периодонтального кармана у пациентов с болезнями периодонта наиболее рациональным является использование бумажного штифта как стоматологического инструмента.

При разработке отечественного экспресс-теста в периодонтологии целесообразным представляется проведение подготовительных клинико-лабораторных мероприятий с использованием ПЦР и оценка трипсиноподобной активности периопатогенной микрофлоры (*Treponema denticola*, *Porphyromonas gingivalis*, *Bacteroides forsythus*).

Ключевые слова: экспресс-диагностика, периодонтология, клинико-лабораторная диагностика, ткани периодонта

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Introduction. Currently, a range of advanced innovative clinical and experimental studies that enable further improvement of diagnosis and prognosis can be justified and implemented in the Republic of Belarus, which reduces the treatment period, decreases the number of visits to the dentist, as well as prolongs and stabilises the remission of dental diseases [1–4].

The new technologies for diagnosis of periodontal diseases in clinical periodontology become of special importance. In this connection, detailed diagnosis indicators for obtaining comprehensive information on the dental status makes it possible to identify the disease at different stages of its development and to plan comprehensive treatment and prevention efficiently [5–7].

In a balanced dynamic system of periodontium, the dental crown surrounding is represented by a diverse microbial environment. The aggressive oral cavity environment includes potential pathogens, possible virulent versions and foreign microorganisms. The negative impact of the dental plaque disturbs, sometimes, the balance between the surrounding of the dental crown and of the dental root, leading to periodontal tissue changes [8].

It should be remembered that the quality of diagnosis depends on the differential methodology of clinical and laboratory examination, as well as on the compliance with the basic principles of microbiological studies in periodontology [9].

The traditional clinical diagnosis of periodontal diseases is based on the clinical dental examination and monitoring of the hygienic status of the oral cavity, gums, periodontal ligament, alveolar bone and the microcirculation conditions. The efficient diagnosis of periodontal diseases includes the determination of the quantitative and qualitative compositions of the oral cavity microflora. Globally, the following laboratory methods are used for identifying periodontal microorganisms: bacteriological, microscopic, immunological and molecular diagnosis, as well as biochemical determination of microorganism fermentation action markers and tissue destruction and inflammation markers [10–14].

Different diagnosis tests are chosen and used depending on the possibilities of early diagnosis prior to the development of disease signs, high prognosis value of the analysis, test availability and its cost effectiveness. It should be mentioned that the majority of the mentioned methods require special laboratory conditions, skilled and trained staff, hard-to-get equipment as well as specific conditions for biomaterial transportation. The duration of laboratory tests can be from several hours (for instance, PCR, provided the samples are transported to the diagnosis laboratory immediately) to several days (culture methods), which results in delayed healthcare, higher numbers of visits, prolonged treatment and higher costs of treatment. Among the tests based on determining the microorganism fermentation action markers, the most widely used is the BANA test, since it can be performed by the dentist in real time. The obvious advantages of this test are its availability and simplicity, high prognosis value of the analysis, minimal invasiveness, as well as high specificity and sensitivity. This scales up the rapid diagnosis opportunities during the visit to the dentist.

The BANA test is based on its unique capacity for rapid identification of periopathogenic gram-negative anaerobic bacteria of the red complex (*P. gingivalis*, *T. denticola* and *B. forsythus).* It should be

mentioned that this test does not allow an accurate quantitative assessment of bacteria, neither it indicates which of the three bacteria is prevailing. However, its advantage lies in that the dentist is able to identify during a short visit of the patient, whether the analysed material contains a diagnostically significant quality of periopathogenic microorganisms (*P. gingivalis*, *T. denticola* and *B. forsythus*), which is very valuable for dental and interdisciplinary diagnosis, prognosis and prevention of dental diseases. At the same time, the BANA test can be used for assessing the efficiency of the actual dental treatment, for example, scaling, root smoothing, use of antimicrobial preparations for treatment of periodontal diseases, oral mucosa diseases, halitosis and other conditions of the oral cavity [15].

At the same time, it is very important to develop and produce a domestic test for rapid diagnosis, prognosis and rational treatment of periodontal diseases. Therefore, the obvious promising lines for development of microbiological diagnosis in dentistry and the development and introduction of screening and rapid tests will enable the dentist to apply them in dental practices on a broad scale.

The goal of the study – to develop a domestic rapid test for periodontology, indicating the procedure for preparatory clinical and laboratory measures using PCR and trypsin-like activity of periopathogenic microflora (*Treponema denticola*, *Porphyromonas gingivalis* and *Bacteroides forsythus*).

Materials (objects) and research methods. The preparatory clinical and laboratory studies were conducted by Chair of Therapeutic Dentistry No. 3 of the Belarusian State Medical University and the Republican Scientific and Practical Centre of Epidemiology and Microbiology [16].

Clinical studies. The researchers performed calibration according to the international standard.

Before the study, the patients gave their voluntary informed consent for diagnosis procedures and personal data processing. Dental examination was performed in 60 patients who had clinical signs of generalized periodontitis and a 5–6 mm deep pathological dentogingival pocket (PDGP), aged 35–44 (the main group), and 140 patients without signs of periodontal diseases, aged 20–24 (the control group) (Tab. 1).

In selecting dental tools for extraction of the content from the dentogingival pocket, PCR diagnosis was used to identify the genetic material of periopathogenic microorganisms, such as *Treponema denticola*, *Porphyromonas gingivalis* and *Bacteroides forsythus*, using specific oligonucleotides (primers) and labelled hybridization samples, a complementary sequence of gens 16S of ribosomal genome RNA of these exciters. Totally, 1260 samples were prepared for PCR diagnosis.

This work was performed using clinical and tool methods for assessing the status of hard tooth tissues, periodontium, oral cavity hygiene, including the simplified oral hygiene index OHI-S (I. G. Green, I. R. Vermillion, 1964), the plaque thickness index (PLI) (J. Silness and H. Loe,1964), the gingival index (GI) (H. Loe, J. Silness, 1963), papillary-marginal-alveolar index (IPMA) (M. Massler, J. Shour, C. Parma, 1960), periodontal index (PI) (A. L. Russel, 1956, 1967), the index of gingival recession (IR) (S. Stahl, A. Morris, 1955), X-ray diagnosis methods, as well as their statistical processing.

First, supragingival dental calculus was removed mechanically; then the content of the gingival sulcus and/or the periodontal pocket was removed in the area of the medial vestibular surface of the first maxillary molar.

Table 1. **Index indicators for assessing the state of periodontal tissues in patients of the main and control groups (M ± m)**

Clinical indicator	Main group	Control group
Probing depth, mm	5.2 ± 0.18	2.7 ± 0.16
OHI-S, score	0.59 ± 0.11	0.57 ± 0.08
PLI, score	0.78 ± 0.15	0.77 ± 0.13
GI, score	1.86 ± 1.28	0.75 ± 0.09
IPMA, %	35.82 ± 2.31	7.57 ± 0.45
PI, score	2.77 ± 1.13	0.09 ± 0.07
IR, %	34.73 ± 2.98	18.95 ± 2.15

For removing the periodontal pocket content, the following sterile tools were used in the study: a dental probe, a dental excavator no. 1, a periodontal probe (WHO), a periodontal probe (North Carolina), a dental brush, and a paper pin. The tool was inserted into the periodontal pocket for 10 sec; then the pins or the biomaterial from the probes, the excavator and the dental brush were placed in a test tube

with the sterile saline solution (150 µl) and sent for freezing and storage pending their transportation to the laboratory.

The laboratory tests. The laboratory diagnosis was made using PCR, and trypsin-like activity of the biological samples was assessed.

DNAs were extracted from the clinical material by means of commercial sets RIBO-prep, Russian Federation, and NK-extra, Republic of Belarus.

The following oligonucleotides (primers) and hybridization samples were used for qualitative and quantitative PCR:

PG-F: GCGCTCAACGTTCAGCCT;
PG-R: CACGAATTCCGCCTGCC;
PG_FAM: FAM-GGCAGTTTCAACGGC-BHQ;
TF-F: TGAAAGTTTGTCGCTTAACGATAAAA;
TF-R: TCGTGCTTCAGTGTCAGTTATACCT;
TF_ROX: ROX-CATTCCGCCTACTTCATC-BHQ2;
TD-F: CTTCCGCAATGGACGAAAGT;
TD-R: CAAAGAAGCATTCCCTCTTCTTCTTA;
TD_By-5: By5-GTAAAATTCTTTTGCAGATGAAG-BHQ2.

The standard samples for quantitative identification of periopathogenic microorganisms (*T. denticola*, *P. gingivalis* and *B. forsythus*) included the recombinant plasmid DNAs: pJET1.2/blunt/PG., pJET1.2/blunt/BF, pJET1.2/blunt/TD, obtained at the previous research stages. Two standard samples (calibrators) were used for each exciter: $3.5 \cdot 10^7$ and $1.3 \cdot 10^3$ gEq/ml for identification and quantitative analysis of *T. denticola*, $2.6 \cdot 10^6$ and $2.6 \cdot 10^3$ gEq/ml for identification and quantitative analysis of *B. forsythus*.

The PCR mix for qualitative and quantitative identification of periopathogenic microorganisms (*T. denticola*, *P. gingivalis* and *B. forsythus*) included the following components calculated per reaction: Primer PG-F (0.5 µM), Primer PG-R (0.5 µM), Probe PG_FAM (0.4 µM), Primer TF-F (0.5 µM), Primer TF-R (0.5 µM), Probe TF_ROX (0.2 µM), Primer TD-F (0.5 µM), Primer TD-R (0.5 µM), Probe TD_By-5 (0.4 µM), dNTP mix (0.2 mM), Taq-buffer (×1), $MgCl_2$ (4 mM), Taq-polimerase (2,5 ME), H_2O (mQ) to have the final volume of the reaction mix 40 µl. The volume of the introduced DNA was 10 µl. The amplification mode: 1. 95 ºC for 3 min; 2. 94 ºC for 15 sec, 54 ºC for 30 sec, 72 ºC for 45 sec, the number of cycles is 45.

The real-time analysis of the amplification results was made by means of the software of the amplification device with the optical module IQ 5 (Bio-Rad, USA). Curves of the fluorescent signal accumulation in the channels FAM, ROX and Cy5 were analysed to record the accumulation of DNA fragment amplification products

The solutions and reagents for biochemical assessment of trypsin-like activity of the pathogen periodontal microflora.

The following types of substrates for proteolytic enzymes were used: N-benzoyl-DL-arginine-β-naphthylamide (BANA) and Nα-benzoyl-DL-arginine-4-nitroanilide hydrochloride (BAPNA). 67 mM solutions of these substrates were used for the reaction. The 0.2 % solution Fast K Black Salt (KP) was used as a dye. The main strip was prepared using a nitrocellulose chromatographic membrane (manufactured by the Glory Science Co., Ltd.) as substrate-impregnated material. The auxiliary strip, impregnated with the dye solution, was prepared using the filter paper according to State Standard GOST 12026-76. The basis for both strips was a PVC sheet with a self-adhesive surface on one side (manufactured by the Glory Science Co., Ltd.).

Results and its discussion. Our clinical experience was used to identify modern clinical and laboratory principles of microbiological diagnosis in periodontology.

***Basic clinical and laboratory principles of microbiological diagnosis in periodontology*:**

selection of the investigation object;
selection of the adequate diagnosis;
selection of the investigation method;
competence of the microbiological diagnosis results in periodontology.

Given the specific characteristics of periodontal tissues, oral cavity microflora and clinical manifestations of periodontal diseases, it is expedient to investigate the content of the gingival sulcus and/or the

periodontal pocket. In this case, it is important to select adequate diagnosis means, since it defines the quality and quantity of the obtained investigation material. Multiple and contradictory data are available that indicate to the lack of a unified methodology for a comprehensive evaluation of the periodontium biological system, including the microbial environment. At the same time, investigation of the periodontal pocket requires high sensitivity and specificity and application simplicity. Analysis of the microbiological study outcomes depends on the level of development of molecular biological methods and the information communication technologies. In this case, the ethical and deontology competences determine the high level of awareness in the issues of microbiology and periodontology and, in general, of the interdisciplinary approach in diagnosis, treatment and prevention of periodontal diseases.

The above enumerated clinical and laboratory principles of microbiological diagnosis in periodontology enable the dentist to obtain true diagnosis results, make correct diagnosis and apply efficient treatment and prevention measures in 98 % of cases.

Thus, we present the results of use of various tools for the extraction of the content from the periodontal pocket by the threshold cycle (Ct) values (Tab. 2).

Table 2. **Selection of rational dental tools for extracting the content from the pathological dentogingival pocket (M ± m)**

The tool for extracting the periodontal pocket content	Threshold cycle (Ct) values in the FAM channel for the studied samples		
	PCR mix 1 *Porphyromonas gingivalis*	PCR mix 2 *Treponema denticola*	PCR mix 3 *Bacteroides forsythus*
Paper pin	24.94 ± 0.23	27.42 ± 0.38	25.76 ± 0.75
Periodontal probe, WHO	34.35 ± 0.32	41.70 ± 0.9	35.71 ± 0.52
Periodontal probe, North Carolina	35.53 ± 0.61	39.05 ± 0.19	35.42± 0.63
Dental probe	36.01 ± 0.13	37.45 ± 0.64	36.38 ± 0.45
Dental brush	31.90 ± 0.73	38.88 ± 0.87	30.27 ± 0.93
Dental excavator	36.65± 0.29	43.57 ± 0.63	32.64 ± 0.82

The results of PCR diagnosis identified the following anaerobic pathogenic microorganisms: *Porphyromonas gingivalis*, *Treponema denticola* and *Bacteroides forsythus*. This reaction is qualitative without identification of the reliable quantity of microorganisms in the investigated samples (by the value of the fluorescence threshold (Ct), obtained for each positive result). Comparing them for one and the same reaction, it can be concluded that the samples with smaller values Ct have higher original quantities of the exciter's genetic material.

The use of all dental tools, included into the research, enabled obtaining a biological material, containing the microorganism DNAs. It should be noted that the greatest quantity of microorganism DNAs was identified in the samples, selected by means of a paper pin. Therefore, we recommend selecting the tools by the decrease of the material DNA: a paper pin, a dental brush (size 0), a periodontal bulbous-end probe (WHO), a periodontal probe (North Carolina), and a dental excavator no. 1.

Qualitative and quantitative PCR analysis of T. denticola, P. gingivalis and B. forsythus in biological samples from PDGP of patients who had periodontitis. For identification and quantitative identification of the genetic material of periopathogenic microorganisms (*T. denticola*, *P. gingivalis* and *B. forsythus*), DNAs were extracted, followed by PCR. The control samples (standards) were the plasmid DNAs obtained at the previous stages of research: pJET1.2/blunt/PG., pJET1.2/blunt/BF, pJET1.2/blunt/TD. The initial number of plasmid DNAs was determined by means of spectrophotometry, followed by recalculation per number of copies by the formula $k = (m \cdot N_A)/1 \cdot 10^9 \cdot 650$, where k is the number of molecules (copies) of the plasmid DNA in the preparation, m is the weight of DNA (ng), N_A is Avogadro constant ($6.023 \cdot 10^{23}$ mol^{-1}, l is the size of the plasmid DNA (bp), 10^9 is the factor of conversion of ng to g, 650 is the molecular weight 1 bp in daltons.

This formula was used to make quantitative calculations for each plasmid DNA with a series of consecutive ten-fold dilutions: from $3.5 \cdot 10^8$ and $3.5 \cdot 10^2$ gEq/ml for pJET1.2/blunt/PG, from $1.3 \cdot 10^8$ and $1.3 \cdot 10^2$ gEq/ml for pJET1.2/blunt/TD, and from $2.6 \cdot 10^8$ and $2.6 \cdot 10^2$ gEq/ml for pJET1.2/blunt/BF.

Thereafter, the obtained plasmid DNAs in the concentrations $3.5 \cdot 10^7$ and $3.5 \cdot 10^3$ gEq/ml for pJET1.2/blunt/PG (*P. gingivalis*), $1.3 \cdot 10^5$ and $1.3 \cdot 10^3$ gEq/ml for pJET1.2/blunt/TD (*T. denticola*) and $2{,}6 \cdot 10^6$ and

$2.6 \cdot 10^3$ gEq/ml for pJET1.2/blunt/BF (*B. forsythus*) were used as standard control samples (calibrators) for quantitative assessment of DNAs *T. denticola*, *P. gingivalis* and *B. forsythus* in the clinical samples taken from the patients. Tab. 3 shows the results of quantitative identification of periopathogenic microorganisms in the clinical samples from the patients; the comparison set was the commercial preparation "Dentoscreen", manufactured by the "Lytech".

Table 3. **Results of quantitative identification of periopathogenic microorganisms by PCR in real time**

Sample nos.	Using an optimized PCR mix and the obtained standard samples (gEq/ml)			Using the commercial preparation "Dentoscreen", manufactured by the "Lytech" (gEq/ml)		
	PG (FAM)	TD (Cy5)	BF (ROX)	PG (FAM)	TD (FAM)	BF (FAM)
34	$1.19 \cdot 10^5$	–	$1.15 \cdot 10^4$	$1.52 \cdot 10^5$	–	$1.27 \cdot 10^4$
35	–	–	$8.92 \cdot 10^1$	–	–	$7.88 \cdot 10^1$
36	–	–	$8.14 \cdot 10^1$	–	–	$7.95 \cdot 10^1$
38	$3.7 \cdot 10^1$	–	–	$4.56 \cdot 10^1$	–	–
39	–	–	$5.06 \cdot 10^3$	–	–	$5.37 \cdot 10^3$
40	–	–	$5.5 \cdot 10^2$	–	–	$3.78 \cdot 10^2$
41	–	–	$9.05 \cdot 10^3$	–	–	$8.43 \cdot 10^3$
42	–	–	$2.77 \cdot 10^2$	–	–	$2.81 \cdot 10^2$
St 1	$3.5 \cdot 10^7$	$1.3 \cdot 10^7$	$2.6 \cdot 10^6$	$1.0 \cdot 10^7$	$3.0 \cdot 10^7$	$8.7.6 \cdot 10^6$
St 2	$3.5 \cdot 10^5$	$1.3 \cdot 10^5$	$2.6 \cdot 10^3$	$7.9 \cdot 10^4$	$1.2 \cdot 10^5$	$2.9 \cdot 10^4$

Comparable results of quantitative identification of DNA of periopathogenic microorganisms were obtained for all investigated samples, both using an optimized PCR mix and the obtained standard control samples and the commercial diagnosis preparation.

The biochemical reaction for detection of trypsin-like ferments in reactions with different synthetic substrates. The basic feature in the development of biochemical/enzyme tests for periodontitis diagnosis is the unique capacity of periopathogenic gram-negative anaerobic bacteria of the red complex, including *P. gingivalis*, *T. denticola* and *B. forsythus*, i. e., the capacity to manifest trypsin-like activity in relation to synthetic derivatives of β-naphthylamide. It was established and demonstrated that the red complex bacteria are capable of hydrolysing a number of compounds (substrates), in particular, N-α-benzoyl-DL-arginine-2-naphthylamide (BANA) and N-α-benzoyl-DL-arginine-p-nitroanilide (BAPNA), generating the final reaction product which can be visualised by introducing the relevant dye. Based on these data, we optimised the biochemical reaction for identification of trypsin-like ferments in the initial investigated material using various substrates. The fermentation reaction was performed in the solution and on different types of membranes. For this reaction, we used 67 mM solutions of N-benzoyl-DL-arginine-β-naphthylamide (BANA) and Nα-benzoyl-DL-arginine-4-nitroanilide hydrochloride (BAPNA) as substrates for proteolytic enzymes. The dye was 0.2 % Fast K Black Salt (KP) solution.

In the reaction using Nα-benzoyl-DL-arginine-4-nitroanalide hydrochloride (BAPNA) as the substrate, the solution manifested a subtle straw change in the modified colour after the introduction of the dye. In this case, the ratio BAPNA/trypsin (2 mg/ml) was 4:1. The reaction products were visualized 5 min later by introducing the dye until a stable colour was obtained. The reaction on a chromatographic membrane did not colour the fermentation reaction area (the control was trypsin from 3 to 10 μg) after incubation with the dye.

During the reaction using N-benzoyl-DL-arginine-β-naphthylamide (BANA) as the substrate, after the introduction of trypsin (3 μg) and subsequent incubation with the dye in the area of substrate fermentative degradation, we recorded a coloured complex of various intensities depending on the substrate used for the auxiliary strip (with the dye). The highest coloration intensity and, hence, the reaction efficiency were observed when the filter paper per GOST 12026-76 was used as the base for the dye impregnation, as compared to the blotting paper P-4681 Sigma and the filter paper BR 7289022 (Bio-Rad, USA).

Afterwards, test strips were prepared that allowed investigating up to five samples at a time. For positive control, trypsin was placed on the membrane with the substrate in the respective region, which was visualized together with the investigated samples after incubation with the dye placed on the auxiliary

strip. Figure 5 shows the results of identification of periopathogenic microorganisms *P. gingivalis*, *T. denticola* and *T. forsythia* in clinical samples taken from patients (the content of the dentogingival pocket) who had clinically diagnosed chronic generalized periodontitis. In this case, the content of the dentogingival pockets, extracted by means of paper pins, was placed directly on the designated reaction area of the main strip.

Following the application of the samples and incubation with the dye in the respective area it was possible to visualize grey-blue strips of various intensities, testifying to the presence of periopathogenic microorganisms in the sample. For positive control, we visualized a grey-blue strip in the reaction control area.

The algorithm of sample preparation and laboratory diagnosis of the periodontal tissues in patients with periodontitis, using a PCR and a biochemical rapid test based on the assessment of trypsin-like activity of biological samples. The algorithm of sample preparation and laboratory diagnosis of periodontitis (hereinafter, the algorithm), using a PCR method and assessment of trypsin-like activity of biological samples, means a sequence of actions that define the procedure of sampling biological material from patients who have periodontitis or who are suspected to develop inflammatory processes related to anaerobic microflora, and its further investigation by means of biochemical method and a real-time quantitative PCR method.

This algorithm includes the following stages:

sampling the material and identifying periopathogenic microorganisms *T. denticola*, *P. gingivalis* and *B. forsythus* in the sample by means of a biochemical method;

sampling the material and its preservation and storing to be analysed by the quantitative PCR;

identifying the genetic material of the periopathogenic microorganisms *T. denticola*, *P. gingivalis* and *B. forsythus* by the real-time quantitative PCR.

The preparation stage includes the mechanical removal of supragingival dental calculus followed by extraction of the content from the gingival sulcus and/or the periodontal pocket in the area of the medial vestibular surface of the first maxillary molar. The periodontal pocket content is removed using sterile dental paper pins. The paper pin is inserted into the periodontal pocket for 10 sec; then it is withdrawn and used for direct biochemical identification of periopathogenic microorganisms *T. denticola*, *P. gingivalis* and *B. forsythus* by means of the biochemical rapid test; and placed to a micro test tube with a sterile saline solution to store at –20 ºC and to be transported to the laboratory for identification of genetic material of the periopathogenic microorganisms *T. denticola*, *P. gingivalis* and *B. forsythus* by means of the real-time quantitative PCR.

Conclusions

1. The basic principles of microbiological diagnosis in periodontology enable making a true diagnosis, planning treatment and assessing treatment results.

2. The competence in selecting the investigation object testifies to the need of studying the content of the gingival sulcus and/or the periodontal pocket in patients who have periodontal diseases. It was noted that the most rational tools for removing the periodontal pocket content in patients who have periodontal diseases was a paper pin.

3. The development of a domestic express test in periodontology demonstrated the need of preparatory clinical and laboratory activities using PCR and trypsin-like activity of periopathogenic microflora (*Treponema denticola*, *Porphyromonas gingivalis*, *Bacteroides forsythus).*

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