RIGA TECHNICAL UNIVERSITY

Faculty of Transport and Mechanical Engineering

Institute of Biomedical Engineering and Nanotechnologies

Alevtīna LEICE

PhD study program "Engineering Technology, Mechanics and Mechanical Engineering"

DYNAMIC TESTING METHOD FOR EVALUATION OF BIOIMPLANT EFFECT ON HEMOTOLOGICAL PARAMETERS

Field: Mechanical engineering
Subfield: Measurement instrumentation and metrology

PhD Thesis summary

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PROMOTION WORK HAS BEEN SUBMITED FOR OBTAINING OF ENGINEERING SCIENCES DOCTOR SCIENTIFIC DEGREE TO

RIGA TECHNICAL UNIVERSITY

Promotion work for obtaining engineering sciences doctor scientific degree will be defended 20.04. 2010 in Riga Technical University faculty of Transport and Engineering Science, to address: Ezermalas street 6, 405 room.

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CONFIRMATION

I confirm, that I have worked out the promotion work submitted for consideration to Riga Technical University to obtain of doctor engineering sciences degree. The promotion work has not submitted to any other university for obtaining of scientific degree.

Alevtīna Leice _____

Date: _____

Contents

SIGNIFICANCE OF RESEARCH

Since the 1960s, the use of biomaterials for the treatment and replacement of damaged organs or their parts has significantly expanded enabling the improvement in the quality of life and prolonging the life-span of patients.

Today, over 40 different materials are used for implants of more than 40 body parts. The number of implantations amounts to 4 -5 million worldwide annually [116].

The rise in the number of implants used in experimental clinical practice has increased the temporary or permanent direct contact with blood, thus demanding further investigation on the influence of new bio-material on blood cell parameters. This type of research is **vitally necessary** will promote the **development of bioimplant technology** [74,112].

The materials chosen in devices and equipment of implantation which directly interact with the biosystem are called biomaterial and they are often made of metal, ceramics, polymers or biocomposite materials [3, 14,101,102,114].

The biomaterial chosen for implants is determined by several and various factors, though the most essential is **biocompatibility**. Biomaterial is considered biocompatible if it is accepted by all the functions in the body and does not cause any problems. [114].

Biomaterial implanted into the body is either perpetually or temporarily in contact with a living organism's ecosystem, including the blood and affecting blood cells. The bioimplant performance may affect the organism, while the implant itself can cause inflammation of adjacent tissues and may be rejected [40, 75, 82,103] The total effect of biomaterial results in essential changes in tissues and cell composition within the body [17, 114].

Implantation always affects the adjacent tissues of the organism [26, 74,114]. Therefore biomaterial has a specific *bioactivity* [9, 10, 16, 86, 99]. This bioactivity can be both harmful to a living organism (clotting, the promotion of hemolysis, inflammation, etc.) and favourable (cases in the activation of osteogenesis – sediments of hydroxiapatitis on the surface of a titanium implant which improve the biological acceptance of the implant [13, 14].

Nowdays with the great variety of bioimplants it becomes a topical issue to ascertain the response reaction of adjacent cells.

Evaluation of the effect of biomaterials is based on *in vivo* and *in vitro* experiments to determine the general impact of biomaterial on the biosystem [20, 116]. The biosystem's response reaction to the bioimplant is investigated using different tests which, depending on the aim of implantation, primarily examine the most important biocompatibility issues in connection with life-threatening conditions for the patient [12, 20, 24, 52, 53, 56, 62, 70]. For

example, the main question in evaluating the effect of blood vessel implants is the development of clots and hemolysis. In cases when an implant is in direct contact with blood within blood vessels themselves, investigation methods are divided into three types [11, 26,112]: *in vitro* static method; *ex vivo* dynamic method; *in vivo* dynamic method.

In turn, there is no any investigation method providing estimation of bioimplants *in vitro* in dynamics. The *in vitro* dynamic method provides a wider spectrum of obtained blood parameters over a shorter period of time which is less expensive.

The moment the bioimplant comes in contact with blood within the body, a biocompatibility reaction occurs. When blood protein is absorbed on the surface of the bioimplant, it reaches its maximum during 60-120 minutes of incubation [5, 112]. Such research data has been published regarding blood protein absorbtion on bioimplants [45, 49, 66, 77, 114, 116] however, the effect of bioimplants and the changes in blood cell haemotological parameters in dynamics have not been evaluated. The absence of such data complicates the evaluation of **early biocompatilibity**.

Taking into consideration the aforementioned, the results of the promotion thesis devoted to the development of the dynamic testing method used to evaluate the effect of bioimplants on blood cell haemotological parameters is **topical** and will enable the assessment of bioimplant **early biocompatibility**.

GOALS AND OBJCTIVES OF THE PAPER

The aim of the study:

1) The purpose of the study was to develop an in vitro dynamic testing method for evaluation of the bioimplant impact on hematological parameters of the peripheral blood cells.

The study tasks:

1) To elaborate methodology for this experimental study.
2) To choose biomplant specimens.
3) To select and prepare blood specimens.
4) To choose a hematology analyzer and the method of application.
5) To detect the repeatibility of experimental variables.
6) To perform *in vitro* experiments aimed at detecting blood cell hematological parameters after blood/biomplant contact and reveal their changes in dynamics.
7) To perform data processing, analysis of variables and statistical comparisons.

SCIENTIFIC NOVELTY AND MAIN RESULTS OF THE RESEARCH

1) **"Dynamic testing method *in vitro* for evaluation of the bioimplant impact on hematological parameters of the peripheral blood cells"** has been developed.
2) Statistically significant changes in hematological parameters of different blood cells are stated to occur already within an hour after collecting blood specimens.
3) It is stated that patient blood **individual differences** affect the experimental variables**,** blood "aging" process **(time factor)** and, besides, these differences influence the blood cell reaction to bioimplant **(bioimplant factor)**.
4) It is revealed how to avoid the impact of blood **individual differences**:
   - by testing both "experimental" and "reference" specimens simultaneously and by collecting blood specimens of the control group from the same persons as the "experimental" specimens.
   - by using the changes in blood cell hematological parameters from baseline values for evaluation of the study results.

PRACTICAL APPLICATION

The proposed method can be used for:

1) Testing bioimplants of different materials;
2) Assessing changes in blood parameters (from the baseline values) occuring over the time of exposure to bioimplants in dynamics (several consecutive times within an hour);
3) Evaluating the bioimplant impact on blood cells in venous blood specimens with the baseline variables different from reference values;
4) Detecting the optimal number of investigations to evaluate the bioimplant impact on hematological parameters.

IN THIS PAPER AUTHOR DEFENDS

Developed dynamic in vitro testing method that will allow to assess the implant's effects on the body's response on blood cells and hematological parameters level and will enable to assess the early biocompatibility of bioimplant and tissue;

PAPER APPROBATION

1) The assessment of the quality of bioimplant material was performed within the framework of the European project PERCERAMICS;
2) The method was used in two research studies at Pauls Stradins Health and Social Care College:
   - „The adhesive properties of blood cells";
   - „The analytical method for evaluation of the affect of titanium and hydroxyapatite ceramic layer".

The results of the promotion thesis were used in teaching programmes at:

1) „Pauls Stradins Health and Social Care College": hematology, coagulogy.
2) Clinical and Diagnostic laboratory Ltd. „BALT INFO LAB": advanced training for laboratory staff and biomedical lab technician.
3) The results were used in five Diploma papers and Thesis for Master's degree.

The results of the promotion thesis are presented:

In 15 publications (7 articles, 8 konference thesis) and at 7 International conferences and at 10 scientific seminars (RTU BINI: 6; Pauls Stradins Medical College, University of Latvia: 2; Eksperimental and Clinical Medical Institute: 1; Red Cross Medical College: 1).

Experimental sites:

1) Riga Technical University, BIN Institute
2) The Research Institute of Cardiology, University of Latvia (Ethics Committee of the Research Institute of Cardiology, University of Latvia for Clinical and Physiological Research, and Drug and Pharmaceutical Product Clinical Investigation
3) Institute of Experimental and Clinical Research
4) Pauls Stradins Medical College, University of Latvia
5) Red Cross Medical College
6) Clinical and Diagnostic laboratory Ltd. „BALT INFO LAB" (Certificate No.L-216) where

   254 venous blood samples were collected. 1280 tests of the peripheral blood were performed and 19200 hematological parameters were studied using these samples.

PUBLICATIONS

1. Leice Alevtina, Dekhtyar Yuri, Britzina Natalya, "Behavior of Blood Cells being in contact with Hydroxyapatite Coated Titanium plate within an hour" 8th International Conference & Workshop, Medical Physics 2010., 14-16 oktobris 2010. Kaunas.
2. Leice Alevtina, Dekhtyar Yuri, Britzina Natalya. "Method to test influence of bio-implants on blood cells by consecutive measurements" "Enviromental and experimental Biology BIOLOGY" – 8.sējuma, 1-4 numurā 2010.
3. Leice A., Dekhtyar Y., Britzina N., Arabere L., Arhipovich V. „Human Blood Cells Affected by Hydroxyapatite Coated Titanium". 14th. IFMBE Proceeding, volume 20, NBC – 14th Nordic-Baltic Conference on Biomedical Engineering and Medical Physics. June 16 – 20, 2008, Riga, Latvia. (*CD Nr. 157)***.**
4. Roth W., Leice A., Dekhtyar Y., Britzina N., Arabere L., Arhipovichs V. „Influence of hydroxyapatite coated titanium on human blood cell" „Biomedical Engineering". – Kaunas University of Technology, 25-26 October 2007, pp 264-267.
5. Leice A., Brūvere R., Gabruseva N., Balodis V. Evaluation of Chernobyl Accident Late Health Effects by Hematological and Clinical Biochemistry tests. Acta Universitatis Latviensis. Medicine, 2001, N 643, p. 76 – 84.
6. Mazarevica G., Freivalds T., Bruvere R., Gabruseva N., Leice A., Zvagule T.. Reactive Properties of separate erythrocytes of Chernobyl clean-up workers at different pH. - SPIE Proceedings. Optical Diagnostic of Living Cells. III, San Jose, USA, 2000, vol. 3921, p. 163 – 171.
7. Mazarevica G., Freivalds T., Bruvere R., Gabruseva N., Leice A., Zvagule T.. Changes in optical properties of erythrocytes at different pH in Chernobyl clean-up workers. - Scientific Problems of Disaster Medicine and Emergency Medical Aid. Proceedings Acad Sci Ukraine. Kiev, Ukraine. 2000, p. 509 – 516.

THE STRUKTURE AND THE SIZE OF THE WORK

The work of promotion is written in latvian language. It contains an introducton, 3 chapters, conclusions, references, attachments, drawings and illustrations. Together 97 pages. The bibliography of 120 titles.

RESEARCH METHOD AND MATERIALS

Research method

The study was performed at a Clinical and Diagnostic Laboratory, and approved by Ethics Committee of the Research Institute of Cardiology, University of Latvia for Clinical and Physiological Research, and Drug and Pharmaceutical Product Clinical Investigation (Protocol No.9, the Meeting of the Ethics Committee, October 6, 2010).

The experimental design of the study was directed towards evaluating the biomaterial impact on blood cell hematological parameters *in vitro* blood tests. In total, **254** venous blood samples [53] were taken from volunteers (aged 18 to 70 years). Blood samples remained anonymous and were used in **1280** tests, and **19200** measurements of blood hematological parameters were obtained.

Venous blood samples were storaged in Vacutainer tubes (Becton-Dickson) with anticoagulant K_2EDTA. Each sample contains 3 ml of blood. Samples of blood between the tests were stored at room temperature ($+18^o$ to $+22^o$ C) and tests were performed within an hour by an ABX Micros OT hematology analyzer.

The analysis was performed at a certified Clinical and Diagnostic Laboratory, Ltd. „BALT INFO LAB". Fifteen blood hematological parameters conventionally used in clinical practice were tested including the count of red blood cells, values of mean red cell volume, red cell distribution width, blood hemoglobin level, mean hemoglobin level in red blood cells, mean cell hemoglobin concentration in red blood cells, hematocrit, the count of platelet, mean platelet volume, platelet distribution width, plateletcrit, the count of white blood cells and the % proportion of white blood cells – granulocytes, monocytes, lymphocytes [1, 31, 34, 35, 36, 39, 40, 43,50,61,63, 84, 85, 93, 96, 98, 108, 111] .

Each of specimens was divided into two parts: reference and experimental samples. The implants were positioned into experimental sample (the ration of bioimplant/blood volume was 1:10). Both experimental and reference samples were tested for several consecutive times in dynamics (the baseline measurement – 0, and four times within an hour: at 10^{th}, 20^{th}, 40^{th} and 60^{th} minutes after start of the experiment).

The factors influencing study results [57, 59] were excluded during the development of the bioimplant [2, 4, 5, 7, 8, 11, 13, 15, 21, 22, 27, 29, 30, 33, 41, 42, 44] testing method:

1. Subjective factor of pre-analytical study phase was excluded using the regulation of blood collecting, storage and preparation;
2. Factors related to analytical phases **[**13, 28**]** were stabilized using automated precise thechnology. *Errors* were evaluated by experimental repeatibility [28, 105];
3. Study factors: blood „aging" *in vitro* and patient blood individual diferences were analysed investigating referent specimens [25, 30, 60, 69, 73, 74, 76, 100].

Bioimplant material and model

The bioimplant material for testing was chosen taking into consideration the increasingly growing number of transplantation cases. The bioimplant model [110] chosen for this study was the titanium fusion **Ti6Al4V,** which was deposited with hydroxyapatite [16, 19, 23, 46, 55, 65, 80, 115, 116.]. The Ti 2mm thick plates (1x2 cm^2) were cut off titanuim tape which was received in the framework of the European project PERCERAMICS.

Statistical treatment of results

The following statistical parameters were used [6]: **Mean value** $(\bar{x})$**; Standard deviation (**SD**); Standard error of mean (**SEM**); Confidence interval (**CI**).**

Statistical evaluations of results were performed using: **analysis of data repeatability, one-way analysis of variance, two-way analysis of variance, linear correlation.**

Statistical comparison of results of reference and experimental blood specimen treatment was done (95% significance).

Evaluation of Repeatability

Venous blood sample which was tested 14 times within an hour by a definite ABX Micros OT hematology analyser was used in the experiment. The repeatability was calculated from the data obtained [28, 105].

Blood sample was colleted from the same person, thus, patient blood individual differences were excluded. Time interval between the measurement cycles were 4 minutes and blood „aging" *in vitro* during a measurement cycle was non significant.

Study regulations were strictly observed keeping permanent room temperature (+18 ... +22° C), a study-tube stirring regimen and speed (8 turnovers/ 8 seconds). Conditions of analytical process during the experiment were constant: the same evaluation methods, callibrators, reference materials and reagents.

The differences in blood sample parameters under these conditions were detected during a short time interval (4 min). Taken into consideration the calculated parameter SD_a, the errors of experiment were within the limits of analyzer manual [28] and they were **non significant,** therefore the method allows to state even minimal changes of blood parameters.

As the variations of blood parameter repeatability during the 4 minute interval was conciderably lower than values of blood parameters, the 4 minute measurements were accepted. Thus, for testing the effect of bioimplant the following intervals: 10, 20, 40 and 60 minutes were chosen.

The influence of time factor

To evaluate the effect of bioimplant on blood cells, it was necessary to form a reference group (for comparison). For this purpose, 111 venous blood samples were collected using Vacutainer tubes with K_2EDTA. Blood samples were tested two times with one hour interval according to the experimental method developed. The referent database (DB.RP) was created.

The effect of time factor on fifteen blood parameters, *Fisher criteria* („*K*") was calculated applying one-way analysis of variance (ANOVA).

Time factor has a statistically significant influence on the blood parameter differences [51, 72, 77, 81, 87, 88, 89,90, 92, 97, 104, 106, 107, 109, 113, 118,119] (DIF.RBC, DIF.HGB, DIF.HCT, DIF.MCV, DIF.RDW, DIF.MPV, DIF.PDW) indicating essential changes in the values during one hour after blood sample collecting and storaging at room temperature (+18 ... +22 ° C).

Prevention of individual influence of blood samples

To judge about the changes in individual blood parameters (from baseline values), the differences in blood sample parameters [DIF] were analysed by the means of histograms. They are shown in Fig. 1 – 3: DIF.RBC, DIF.PLT and DIF.WBC, as an example. Two

vertical lines in histograms represent Standard deviation limits (SD_a) of blood parameter repeatability.

Dispersion of blood parameters was compared with Standard deviation (SD_a). The analysis of hystograms (Fig. 1-3.) reveal the fact that blood parameter variations are not related to errors during testing process in the analyzer.

To obtain additional arguments for the fact that results obtained are not due to testing errors, **linear** correlation coefficients between the changes in the count of three blood cell types were calculated (Table 1.). The correlation proves to be statistically significant. This indicates that changes were related to blood cells *per se*, because the processing of them was methologically independent.

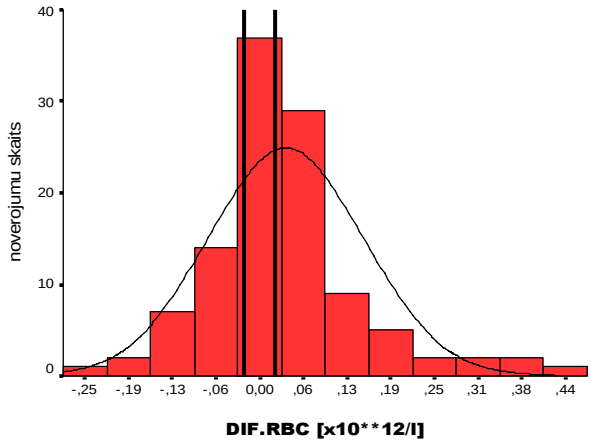

Fig.1. **DIF.RBC histogram**

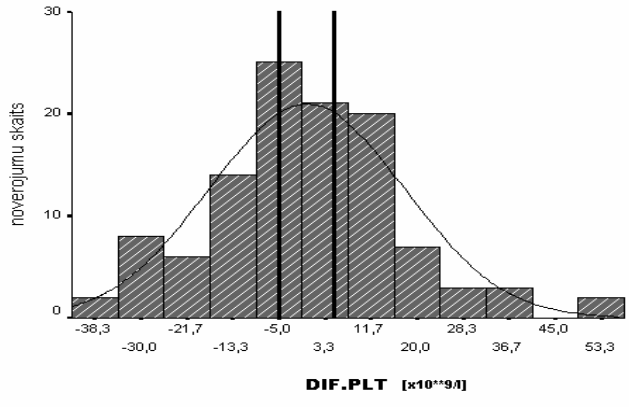

Fig.2. **DIF.PLT histogram**

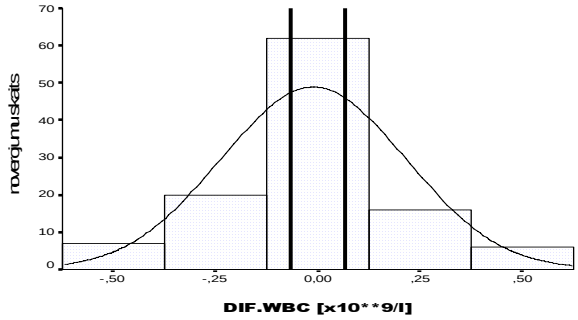

Fig. 3. **DIF.WBC histogram**

Table 1.

Correlation between the changes in three blood parameters

Differences in blood parameters [DIF]	Ststistical values	DIF.RBC [10^{12}/l]	DIF.PLT [10^{9}/l]	DIF.WBC [10^{9}/l]
DIF.RBC [10^{12}/l]	***r***	1	**0,248(**)**	**0,319(**)**
	p-value	.	0,009	0,001
	N	111	111	111
DIF.PLT [10^{9}/l]	***r***	0,248(**)	1	**0,361(**)**
	p-value	,009	.	0,000
	N	111	111	111
DIF.WBC [10^{9}/l]	***r***	0,319(**)	0,361(**)	1
	p-value	0,001	0,000	.
	N	111	111	111

** Correlation is statistically significant ($p < 0.01$).

Close positive correlation ($p < 0.01$) between the difference in the count of red and white blood cells (DIF.RBC un DIF.WBC), red blood cells and platelet (DIF.RBC un DIF.PLT) as well as white blood cells and platelet (DIF.WBC un DIF.PLT) was found (Table 1). This proves that consistent changes in blood parameters can be evoked by storaging samples for an hour *in vitro* at a room temperature independendly of methodological approach but they were related to blood sample individual characteristics.

To prevent the influence of blood sample **individual characteristics** on bioimplant effect, testing of experimental and referent blood samples has to be performed simultaneously. Control group samples have to be collected from the same person blood. In addition, the analysis of changes in blood parameters from the baseline values has to be performed. The baseline values (blood parameter individual diferences) are taken to be zero allowing to eliminate the effect of individual diferences.

THE EFFECT OF BIOIMPLANT MODEL ON BLOOD CELL PARAMETERS

Titanium implant deposited with hydroxyapatite

Venous blood samples of 70 volunteers (males and females) were collected in Vacutainer tubes with K_2EDTA and investigated according to the aforementioned experimental method and equipment and 15 blood parameters were detected.

The initial test values were marked with an index „0". For example, RBC_0 is the count of red blood cells at the baseline measurement. After gentle stirring of Vacutainer tubes, collected specimens were divided into two parts. One portion represents an experimental blood specimen (EP) where the bioimplant is positioned, but the other portion remains as a reference (control) specimen (RP)

Blood samples were stored at a room temperature for 10 minutes and then 15 hematological parameters were detected for both groups, namely, reference and experimental specimens. Now the measurements got test index „10" (RBC_{10}, WBC_{10}, HGB_{10} utt.). Blood parameter testing were similarly performed after 20, 40 and 60 minutes of incubation/storage periods and marked with indexes, respectively, „20", „40", „60". The measurements obtained served as a database (DB.TEST).

Mean values for each blood parameter at every test and for both reference and expererimental samples were calculated with confidence interval 95%.

Time-dependent increase for each blood parameter was calculated by comparing the obtained parameters with baseline values. This difference („DIF") was found out by subtracting test measurements from the baseline value, for example, an increase in the count of red blood cell $DIF.RBC_{10} = RBC_{10} - RBC_0$, i.e., the count of red blood cells 10 minutes after the initial measurement (RBC_{10}) is subtracted from the baseline count of red cells (RBC_0). The differences in other measurements were calculated likewise:

$$DIF.RBC_{20} = RBC_{20} - RBC_0$$

$$DIF.RBC_{40} = RBC_{40} - RBC_0$$

$$DIF.RBC_{60} = RBC_{60} - RBC_0$$

In this case $DIF.RBC_0 = RBC_0 - RBC_0 = 0$. Differences were calculated for all 15 blood parameters. Two factors were found to be involved into the changes of blood parameters —**time factor** and **bioimplant factor**. Therefore, two-way analysis of variance was used for absolute parameter values as well as for differences in parameter measurements

as dependent variables, and „**time”** („K” factor) and **„bioimplant”** („B” factor) factors served as independent values in both calculations.

Testing time („K” factor) according to study design was divided into classes and each class possesses its value which equals to the time interval between the test measurement time and the initial test time (the initial value „0”) of the collected specimens from each person:

„10” – the measurement performed 10 minutes after the start of the experiment;

„20” – the measurement performed 20 minutes after the start of the experiment;

„40” – the measurement performed 40 minutes after the start of the experiment;

„60” – the measurement performed 60 minutes after the start of the experiment.

Bioimplant („B” factor) has two levels: „0” – referent blood sample, „1” – experimental blood sample with the bioimplant plate.

Table 2.

Effect of **time factor** (K) and *bioimplant* **factor** (B) on changes in blood parameters.

Two-way analysis of variance for **DIF**

DIF		“K” factor			“B” factor	
	n		***F***	***p***	***F***	***p***
DIF.RBC	700		3,05	**0,016**	6,72	**0,010**
DIF.HGB	700		3,3	**0,010**	6,5	**0,011**
DIF.HCT	700		1,48	0,206	4,27	**0,039**
DIF.MCV	700		1,46	0,212	3,02	0,083
DIF.MCH	700		2,45	**0,044**	0,041	0,840
DIF.MCHC	700		2,396	**0,049**	0,250	0,617
DIF.RDW	700		11,9	**0,000**	0,048	0,827
DIF.PLT	700		0,41	0,800	12,9	**0,000**
DIF.PCT	700		2,3	**0,054**	6,9	**0,009**
DIF.MPV	700		11,2	**0,000**	0,526	0,469
DIF.PDW	700		0,62	0,648	0,26	0,611
DIF.WBC	700		0,7	0,557	0,10	0,752
DIF.LY	698		15,2	**0,000**	14,5	**0,000**
DIF.MON	698		20,3	**0,000**	20,6	**0,000**
DIF.GRA	698		7,843	**0,000**	4,643	**0,032**

The results obtained by two-way analysis of variance of absolute values of blood parameters indicate non significant changes ($p > 0.05$) referring both to **time factor** and ***bioimplant factor***. Whereas two-way analysis of variance for differences in blood parameters (DIF) reveals statistically significant effect of both factors (**time factor** and **bioimplant factor)** on several blood parameters (Table 2). For example, Fig. 4 -11 illustrate dynamics of

blood parameter DIF in EP and RP specimens during the study period of an hour. Standard error of mean (SEM) was calculated for each blood sample DIF taking into consideration 5 test measurements of EP and RP blood samples separately. The SEM obtained was doubled (according to mathematical standard of error summing) and depicted in Figures:

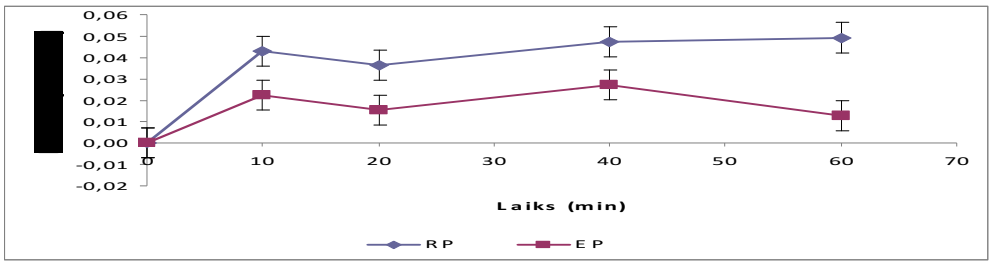

Fig.4. The changes of red blood cell difference (DIF) in RP and EP blood samples

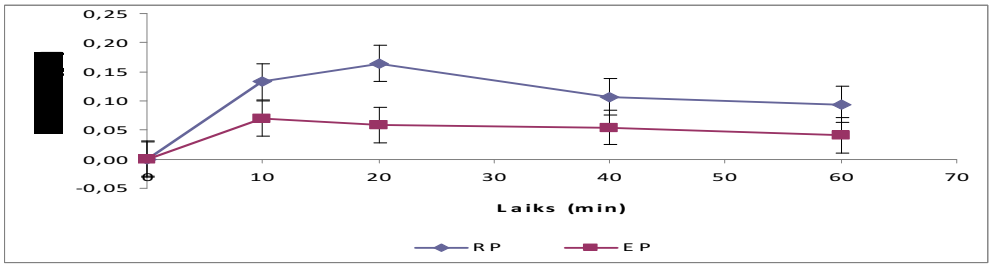

Fig.5.The changes of difference of hemoglobin level (DIF) in RP and EP blood samples

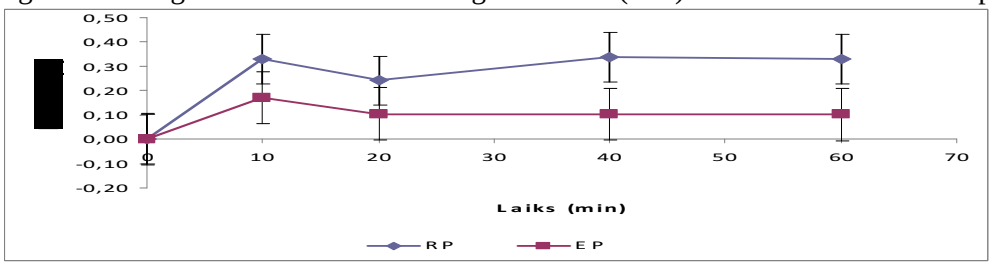

Fig.6.The changes of difference (DIF) of HCT values in RP and EP blood samples

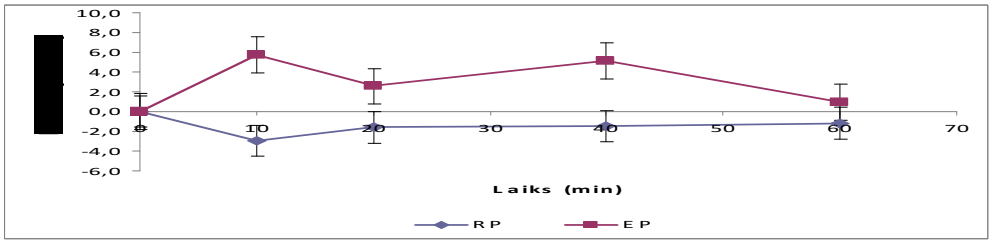

Fig.7. The changes of difference (DIF) of the count of platelet in RP and EP blood samples

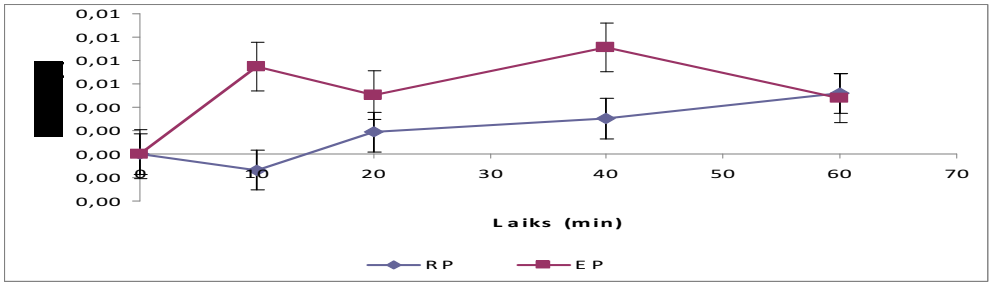

Fig.8.The changes of difference (DIF) of PCT values in RP and EP blood samples

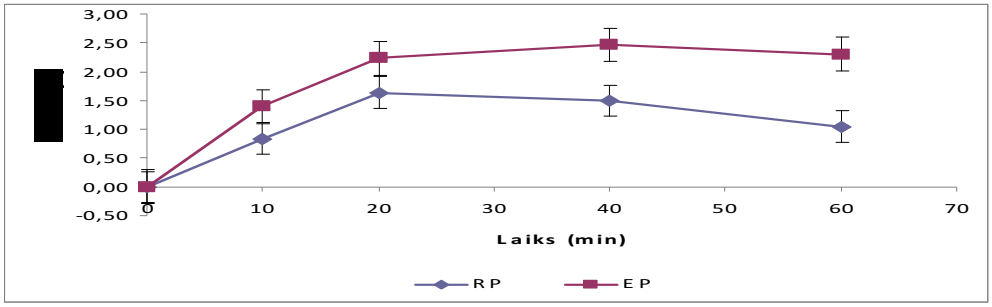

Fig. 9.The changes of difference (DIF) of lymfocyte % in RP and EP blood samples

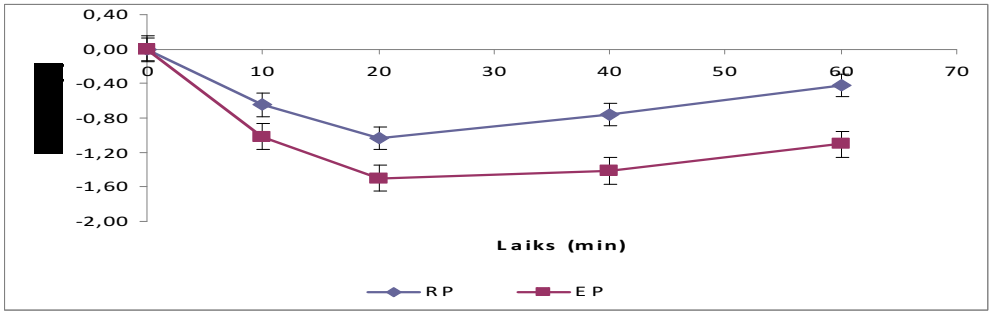

Fig.10. The changes of diference (DIF) of monocyte % in RP and EP blood samples

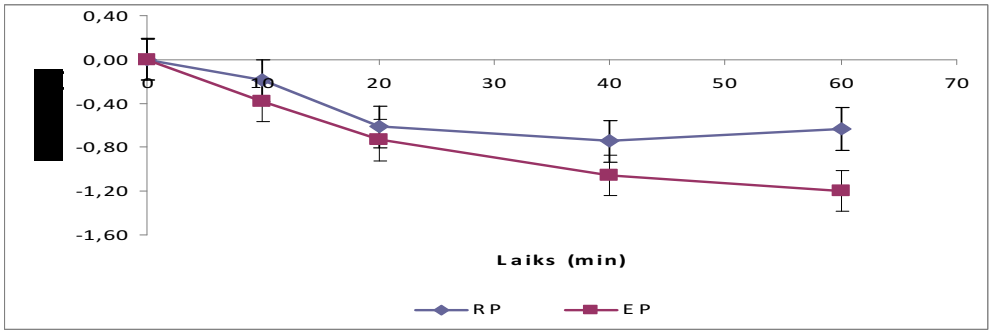

Fig.11.The changes of difference (DIF) of granulocyte % in RP and EP blood samples

Evaluating the difference in blood parameters (DIF) in EP and RP blood samples, it could be concluded that just this difference in EP and RP samples demonstrantes the substantial effect of the bioimplant (Ti-HAP) on blood parameters: DIF.RBC (p <0.010), DIF.HGB (p <0.011), DIF.HCT (p <0.039), DIF.PLT (p <0.001), DIF PCT (p <0.009), DIF.LY (p <0.001), DIF.MON (p <0.001) and DIF.GRA (p <0.032).

The changes in the count of platelet were significantly different in reference and experimental samples. This indicates they were evoked by the bioimplant (Ti-HAP) and could occur due to disaggregation process of platelets or the bioimplant evoked platelet antiaggregation, or because of partly degenerated red blood cells (3-4 times the size of platelets), and these small particles were counted as platelets by analyzer. This process could be related to blood cell/hydroxyapatite contact because hydroxyapatite is known as bioactive [16].

There were observed pronounced changes in the count of red blood cells. Judging by two-way analysis of variance, the count of DIF.RBC is time-dependent both in experimental and reference specimens. But the increase in the count of red blood cells depends on blood cell contact with the bioimplant which delays this process: **bioimplant factor** has statistically significant effect ($p < 0.010$).

The data observed coincide with the results obtained during the study where the stiffness of red blood cell membrane was measured by a atomic force microscopy [71]. The results of this investigation testify that red cell membrane became „harder" if Ti-HAP plate was positioned into the blood sample. This shows that **red blood cell membrane is treated by the contact with the bioimplant.**

White blood cells (the greater in size among blood cells) seem to be inert against **time** ($p = 0{,}557 > 0.05$) and **bioimplant factors** ($p = 0{,}752 > 0.05$). The changes in white blood cell subpopulation % were time-dependent (**time factor** $p < 0.001$ for DIF.LY, DIF.GRA and DIF.MON). The increase in the count of smaller white blood cell — DIF.LY was followed by the decrease in the count of white blood cell of bigger size (DIF.GRA and DIF.MON subpopulations). This agrees with the literature data [76]. This process is substantially affected by blood cell/bioimplant contact (**bioimplant factor** $p < 0.001$ for DIF.LY and DIF.MON, and $p < 0.032$ for DIF.GRA).

The decrease of monocyte size up to lymfocyte size results in changes in automated recognizing and classification of blood cells. This can be explained by blood cell recruitment in subpopulation group of smaller white blood cells. Dynamic changes in lymphocytes and monocytes of white blood cell subpopulation are closely related to **time factor** ($p < 0.001$) but their intensity is modulated by the presence of the bioimplant ($p < 0.001$).

Verification of testing times depending on the number of tests

70 blood samples were tested 5 times within an hour and 15 blood parameters were detected. Analysis of variance was used to evaluate the effect of **time factor** („K" factors) and **bioimplant factor** („B" factor) and *p*-values were calculated (Table 3). The analysis of variance was repeated 4 times in different combination of blood testing times. There were used the following combinations of testing time intervals:

1) Combination of two tests (baseline test and 60 minutes after the start of the experiment);

2) Combination of three tests (baseline test and 40 and 60 minutes after the start of the experiment);
3) Combination of four tests (baseline test and 20, 40 and 60 minutes after the start of the experiment);
4) Combination of five tests (baseline test and 10, 20, 40 and 60 minutes after the start of the experiment);

The results of a 2-test combination performed with an interval of an hour show greater

difference between blood parameters of referent and experimental groups (in contrast to a 5-test combination). This could be explained by a maximum time of exposure of the bioimplant to blood whereas the test number is the lowest.

In case with a 5-test combination, the test number is the highest for statistical analysis while the difference between the test results is the smallest because of the short intervals betwen the tests (the time diference is shorter). *P*-values obtained using two-way analysis of variance characterizing **time factor** („K" factor) and **bioimplant factor** („B" factor) show the essential effect of these factors on 15 parameters.

This is reflected in table 3. If $\boldsymbol{p < 0.05}$, the effect of the factor is considered as significant. The factor effect increases with the decrease in significance (*p*-value). At $\boldsymbol{p > 0.05}$, the factor effect was not found.

Table 3 reveals *p*-values calculated using two-way analysis of variance and characterising the difference in blood parameters. It is shown that *p*-value characterising the bioimplant effect is the lowest at a 5-time testing and the highest at a 2-time testing (an hour interval between the tests). *P*-value is the lowest for **time factor** in case the analysis includes a 2-time test: the results of the baseline test and values obtained at the end of the experiment (after 60 minutes).

The most precise results of the bioimplant effect can be obtained by a 5-time test, whereas two-way analysis of variance for a 2-time test (interval 60 minutes) gives the best picture of time factor effect on blood parameters.

The results of two-way analysis of variance allow to conclude that combinations with different testing time intervals do not exhibit similar sentivity against **time factor** and **bioimplant factor.**

Table 3.

Time („K") and ***bioimplant („B") factor*** effect on blood parameters of Ti:HAP and control blood samples at different combinations of testing time intervals. Two-way analysis of variance for DIF.

Blood parameters (dependent variable)	Test factors (independent variable)	Combinations of different testing time intervals (***p***-values of independent variable)			
		0. and 60. min. tests	0., 40. and 60. min. tests	0., 20., 40. and 60. min. tests	0., 10., 20., 40. and 60. min. tests
DIF.RBC [10 ^{12}/l]	***„K"*** *factor*	**0.001**	**0.001**	**0.006**	**0.016**
	„B" *factor*	**0.049**	**0.030**	**0.016**	**0.010**
DIF.HGB [g/l]	***„K"*** *factor*	**0.008**	**0.018**	**0.007**	**0.010**
	„B" *factor*	0.297	0.159	**0.023**	**0.011**
DIF.HCT [%]	***„K"*** *factor*	**0,013**	0,062	0,160	0,206
	„B" *factor*	0,187	0,077	**0,058**	**0,039**
DIF.MCV [μm^3]	***„K"*** *factor*	**0,003**	**0,028**	0,109	0,212
	„B" *factor*	0,244	0,115	0,106	0,083
DIF.MCH [pg]	***„K"*** *factor*	0,182	0,432	**0,019**	**0,044**
	„B" *factor*	0,260	0,439	0,866	0,840
DIF.MCHC [g/dl]	***„K"*** *factor*	0,942	0,982	**0,019**	**0,049**
	„B" *factor*	0,346	0,790	0,673	0,617
DIF.RDW [%]	***„K"*** *factor*	**0,001**	**0,001**	**0,001**	**0,001**
	„B" *factor*	0,848	0,870	0,849	0,827
DIF.PLT [10 ^{9}/l]	***„K"*** *factor*	0,937	0,506	0,727	0,800
	„B" *factor*	0,511	**0,057**	**0,017**	**0,001**
DIF.PCT [%]	***„K"*** *factor*	**0,006**	**0,012**	**0,029**	**0,054**
	„B" *factor*	0,915	0,285	0,147	**0,009**
DIF.MPV [μm^3]	***„K"*** *factor*	**0,001**	**0,001**	**0,001**	**0,001**
	„B" *factor*	0,143	0,276	0,198	0,469
DIF.PDW [%]	***„K"*** *factor*	0,296	0,606	0,704	0,648
	„B" *factor*	0,191	0,446	0,359	0,611
DIF.WBC [10 ^{9}/l]	***„K"*** *factor*	0,376	0,475	0,697	0,557
	„B" *factor*	0,670	0,942	0,836	0,752
DIF.LY [%]	***„K"*** *factor*	**0,001**	**0,001**	**0,001**	**0,001**
	„B" *factor*	**0,010**	**0,001**	**0,001**	**0,001**
DIF.MON [%]	***„K"*** *factor*	**0,001**	**0,001**	**0,001**	**0,001**
	„B" *factor*	**0,001**	**0,001**	**0,001**	**0,001**
DIF.GRA [%]	***„K"*** *factor*	**0,001**	**0,001**	**0,001**	**0,001**
	„B" *factor*	0,105	0,070	0,090	**0,032**

The effect of the bioimplant on blood with lowered hemoglobin level

In the previous chapters of the thesis it was stated that changes occur in hematological parameters if blood samples are stored for an hour *in vitro* at room temperature. Blood cells react differently if blood cells are in contact with bioimplant.

Table 4.

Significant differences in blood sample parameters between Ti:HAP (***„B" factor***) and control treatments in blood with lowered HGB level **(HGB ≤ 12.5 g/dl)**. Analysis of variance for DIF of 15 blood parameters at a 5-time testing.

Blood parameters (dependent variable)	Test factors (independent variables)	***p*-values of independent factor variables**
DIF.RBC [·10 ^{12}/l]	***„B" factor***	**0,053**
DIF.HGB [g/l]	***„B" factor***	**0,048**
DIF.HCT [%]	***„B" factor***	**0,090**
DIF.MCV [μm^3]	***„B" factor***	0,540
DIF.MCH [pg]	***„B" factor***	0,629
DIF.MCHC [g/dl]	***„B" factor***	0,784
DIF.RDW [%]	***„B" factor***	0,357
DIF.PLT [·10 ^{9}/l]	***„B" factor***	**0,020**
DIF.PCT [%]	***„B" factor***	**0,029**
DIF.MPV [μm^3]	***„B" factor***	0,154
DIF.PDW [%]	***„B" factor***	0,070
DIF.WBC [·10 ^{9}/l]	***„B" factor***	0,524
DIF.LY [%]	***„B" factor***	**0,020**
DIF.MON [%]	***„B" factor***	**0,008**
DIF.GRA [%]	***„B" factor***	0,283

It could by suggest that blood with parameters different from referent values could contain blood cells with altered membrane penetrability affecting the response to the implant. To ascertain this assumption, evaluation of the changes in blood parameters were performed in dynamics *in vitro* in blood sample with lowered hemoglobin level.

A group of blood samples (20 specimens) from patients with frequent pathology (lowered hemoglobin level ≤ 12.5 g/dl) was tested. According to the developed dynamic testing method, 15 blood parameters were measured at the start and then at 5 consecutive times during the experiment in agreement with the study design.

Each blood sample was devided into two portions: referent and experimental portion. Titanuim specimens deposited with hydroxyapatite were positioned into experimental blood samples. All blood samples were tested at the 10^{th}, 20^{th}, 40^{th} and 60^{th} minute after the start of the experiment. For statistical comparisons two-factor analysis of variance was used.

Table 4 shows *p*-values of **bioimplant factor** in the group of blood samples with lowered hemoglobin level being in contact with Ti:HAP plate.

1. There were observed a statistically significant response to the bioimplant in blood samples with lowered hemoglobin level being in contact with Ti-HAP: DIF.RBC (p = 0.053), `DIF.HGB (p = 0.048), DIF.HCT (p = 0.090), DIF.PLT (p = 0.020), DIF.PCT (p = 0.029), DIF.LY (p = 0.020) un DIF.MON (p = 0.008).

2. The developed dynamic testing method *in vitro* was stated to be used as a useful tool for evaluation of the effect of the bioimplant not only in normal blood samples but also in blood samples with hematological parameters different from referent values.

Titanium implant

Blood sample group (16 blood specimens) was used to test the effect of the titanium implant (HAP-free) on blood parameters. According to the developed dynamic testing method, 15 blood parameters were measured at the start of the experiment.

Each blood sample was devided into two portions: reference and experimental samples. The titanium plate was positioned into experimental blood samples. All blood samples were tested 10, 20, 40 and 60 minutes after the start of the experiment. For statistical comparisons one-way analysis of variance (ANOVA) was used.

Substantial effect of bioimplant (Table 5) is found in values of red blood cell parameters – DIF.HGB (p< 0,021), DIF.HCT (p < 0,053), DIF.MCV (p < 0,003) and DIF.MCH (p < 0,029).

The fact that the titanium bioimplant exhibits pronounced effect only on the parameters of red blood cells is in accordance with literature data confirming the titanium bioimplant possesses lower bioactivity in comparison with the titanium deposited with hydroxyalatite.

The results obtained during the study testify that the dynamic testing method developed for evaluation of blood cell parameters *in vitro* can be used for testing different bioimplants.

Table 5.

Comparison of RP and EP samples (HAP- free)

One-way analysis of variance

Blood parameters	***n***	*Fisher statistic*	*Significance (p-value)*
DIF.RBC [10^{12}/l]	158	2,375	0,125
DIF.HGB [g/l]	158	5,406	**0,021**
DIF.HCT [%]	158	3,799	**0,053**
DIF.MCV [μm^3]	158	9,200	**0,003**
DIF.MCH [pg]	158	4,829	**0,029**
DIF.MCHC [g/dl]	158	0,739	0,391
DIF.RDW [%]	158	0,136	0,713
DIF.PLT [10^9/l]	158	0,018	0,895
DIF.PCT [%]	158	0,001	0,971
DIF.MPV [μm^3]	158	0,366	0,546
DIF.PDW [%]	158	0,135	0,713
DIF.WBC [10^9/l]	158	0,069	0,792
DIF.LY [%]	158	3,556	0,061
DIF.MON [%]	158	1,756	0,187
DIF.GRA [%]	158	1,533	0,218

Dynamic testing method

The influence of different bioimplants on blood parameters was evaluated in *in vitro* experiments and procedure of dynamic testing method was developed.

CONCLUSIONS

1. **„Dynamic testing method for evaluation of bioimplant effect on hemotological parametrs”** has been developed.

- According to the developed testing method 254 venous blood specimens were collected. Peripheral blood tests (1280) were performed using these samples and 19200 hematological parameters were investigated. Fifteen blood parameters were detected in each blood sample. Measurements were significant at $p < 0.05$.
- It was stated that to exclude the influence of blood *individual differences* on blood hematological parameters investigating the bioimplant effect, referent and experimental specimen groups should be formed. Moreover, blood samples of the control group should be taken from the same persons as the experimental samples and tested simultaneously.
- It was detected that blood hematological parameters should be controlled by differences in blood characteristics from *baseline values*.
- It was clarified that statistically significant *changes* in various blood parameters already occur *within the first hour* after blood collecting. This means that the optimal length of the experiment should be an hour with at least 5 consecutive measurements for 15 blood parameters providing adequate number of measurements for statistical analysis alongside the bioimplant contact with blood should be at maximum duration.

2. The method *was found to be used for evaluation of the effect of different material implants*. For example, the Ti-HAP implant affects the following blood parameters: RBC, HGB, HCT, PLT, PCT, LY , MON and GRA, but the titanium implant affect: HGB, HCT, MCV, MCH.

3. It was stated that the method can be used for evaluation of *early bioimplant biocompatibility in vitro* not only for samples with normal blood parameters but also for samples with blood parameter values different from reference level used in clinical practice. For example, the effect of the bioimplant on blood samples with low hemoglobin level was found to evoke changes in the following blood parameters: RBC, HGB, HCT, PLT, PCT, LY and MON.

4. The Ti-HAP bioimplant provokes platelet antiaggregation and protects red blood cells. Under the effect of the bioimplant % division of white blood cell subpopulation *in vitro* within an hour was found to change significanly ($p < 0.05$).

Abbreviations and symbols

DIF. RBC	Difference in red blood cell count from baseline value
DIF. WBC	Difference in white blood cell count from baseline value
DIF. LY	Difference in Lymphocyte count from baseline value
DIF.GRA	Difference in Granulocyte count from baseline value
DIF.MON	Difference in Monocyte count from baseline value
EDTA	Ethylenediaminetetraacetic acid
F	*Fisher* statistic
fl	Femtolitre (volume measurement, 1fl = 10^{-15} l)
GRA%	Granulocyte (%)
GRA#	Granulocytes (absolute count, 10^{9}/l)
HAP	Hydroxyapatite
HCT	Hematocrit (%)
HGB	Hemoglobin (g/l)
MCH	Mean red cell hemoglobin level (pg-pikograms)
MCHC	Mean red blood cell hemoglobin concentration (g/dl)
MPV	Mean platelet volume (μm^3)
LY%	Lymphocyte (%)
LY#	Absolute number of lymphocytes (10^9/l)
MON%	Monocyte (%)
MON#	Monocytes (absolute count, 10^9/l)
p	Statistical significance level
PDW	Platelet distribution width (%)
PCT	Plateletcrit (%)
PTL	Platelets (10^{9}/l)
RBC	Red blood cells (10^{12}/l)
RDW	Red cell distribution width (%)
SD	Standard deviation
WBC	White blood cells (10^9/l)

Reference

1. Aberberga-Augškalne L., Koroļova O. Fizioloģija ārstiem. – Rīga: Medicīnas Apgāds, 2007. – 516 lpp.
2. Addonizio VP. Platelet function in cardiopulmonary bypass and artificial organs. *Hematol Oncol Clin North Am.* 1990; 4: 145–155.
3. Alehander W. and Street A., Metals in the Service of Man, 11th edition, New York. Penguin, 1998.
4. Alexiewicz J., *et al*. Impaired phagocytosis in dialysis patients. Studies on mechanisms. *Am. J. Nephrol*., 1991 – 11 – p. 102–111.
5. Andrade J.D., Hlady V. Protein adsorption and material biocompatibility; A tutorial review and suggested hypotheses. *Advances in Polymers Science*, 1986. Vol. 79. p. 1–63.
6. Arhipova I., Bāliņa S. Statistika ekonomikā un biznesā. Risinājumi ar SPSS un MS Excel. – Rīga: Datorzinību centrs, 2006. – 364 lpp.
7. Averbukh Z. et.al., Red Eye Syndrome: Clinical and Experimental Experience in a New Aspect of Diffuse Eosinophilic Infiltration. Artif. Organs, 25, 2001. p. 437-440.
8. Biological Evaluation of Medical Devices, Part 4: Selection of Tests for Interactions With Blood, ISO 10993-4, 2nd Edition; Biological Evaluation of Medical Devices, Part 12: Sample Preparation and Reference Materials, ISO 10993-12, 2nd Edition Corrected version 2003-03-01.
9. Biomaterials, artificial organs and tissue engineering, Edited by Larry L. Hench and Julian R. Jones, Imperial College London, UK, 2005.,304 p.
10. Bonucci E.,Marini E., Valdinucci F., Fortunato G. Osteogenetic response to hydroxyapatite-fibrin implants in maxillofacial bone defects. // *Europ.J.Oral.Sci*. – 1997. – 105, No. 6. – p.557–561.
11. Brian A. Koeneman, Kee-Keun Lee, Amarjit Singh, Jiping He, Gregory B. Raupp, Alyssa Panitch and David G. Capco. “An ex vivo method for evaluating the biocompatibility of neural electrodes in rat brain slice cultures” Journal of Neuroscience MethodsVolume 137, Issue 2, 30 August 2004, p. 257-263
12. Cannas M., Denicolat P., Webb L.X., Gristina A.G. Bioimplant surfaces: Binding of fibronection and fibrolast adhesion. // *J.Orthop. Res*. – 1988. – 7. – p. 316–325.
13. Chen Z.,Chen Y.K., Huang H.H. Blood Coagulation on Titanium Surface with Different TiO2 Crystal Struktures. 23 European Conference on Biomaterials, 11-15th September, 2010, Tampere. FI.
14. Clupper D.C., Hench L.L.and Mecholsky J.J."Strength and toughness of tape cast bioactive glass 45S5 following heat treatment" *Journal of the European Ceramic Society*, 2004. **24**, 2929-2934.

15. Coleman D.L., Lim D., Kessler T., Andrade Y.D. Calcification of nontextured implantable blood pumps // *Trans. Amer. Soc. Artif. Intern. Organs*, 1981. – Vol. 27. – p. 97–103.
16. Courteney-Harris R.G., KayserM.V., Downess S. Comparison of the early production of extracellular matrix on dense hydroxyapatite and hydroxyapatite-coated titanium in cell and organ sulture.// *Biomaterials*. – 1995. – 16, No.6. – p.489–495.
17. Curtis A.S.B., Clark P. The effect of topographic and mechanic properties of materials on cell behavior. // *Crit. Rev. Biocompatibility*. – 1990.– No 5.– p. 362–374.
18. de Gruyter W. Clin Chem Lab Med – 2003 – Berlin – 884. lpp.
19. Deb, S., Giri, J., Dasgupta, S., Datta, D. & Bahadur, D., Synthesis and characterization of biocompatible hydroxyapatite coated ferrite. Bull. Mater. Sci.,Vol. 26, No. 7, December 2003, pp. 655–660.
20. Exbrayat P., Couble M.L., Magloire H., Hartmann D.J. Evaluation of the biocompatibility of Ni-Cr-Mo dental alloe with human ginigival explant culture in vitro: morphological study, immunodetection of fibronectin and collagen production. // *Biomaterial*. – 1987. – 8. – p 385–392.
21. Fishbein M.S., Levy R.J., Ferrans V.J. *et al*. Calcification of cardiovalve bioprostheses: bioprotheses, histological and ultrastructural observations in a subcutaneous implatation model systems // *J. Thorac. Cardiovasc. Surg.*, 1982. – Vol. 82. – p. 602–609.
22. Fogh-Andersen N., Christiansen T.F. et al. Measurement of free calcium ion in capillary blood and serum // *Clin. Chem.*, 1978. – Vol. 24. – p. 1545–1552.
23. Gabrielli, M. A. C., Marcantonio Junior, E., Goissis, G., Okamoto, T., Gabrielli, M. F. R. & Vieira E.H**.,** Hydroxylapatite Implants with or without Collagen in the Zygomatic Arch of Rats. Histological Study. Braz. Dent. J. 2001, 12(1) pp. 9-15
24. Groth T., Atlankov G. Studies on ctll-biomaterial interaction: role of tyrosine phosphorylation during fibroblast spreading on surfaces varying in wettability. // *Biomaterial*.– 1996. – 17, No 12.– p 1227–1234.
25. Gulati G., Hyland L. Changes in automated complete blood cell and differential leukocyte count results induced by storage of blood at room temperature // *Arch patted, lab Med*. – 2002 – 126(3), p. 42–336.
26. Handbook of Biomaterial Properties / Jonathan Black and Garth Hasting. – CHAPMAN HALL, 1998; p. 590.
27. Harasaki H., McHaron J., Richard T. *et al*. Calcification in cardiovascular implants: degraded cell related phenomena // *Trans. Amer. Soc. Artif. Intern. Organs*, 1985 – Vol. 31. – p. 489–494.
28. Hematoloģijas analizators ABX Micros OT, Manuals, Invitros SIA. Lietotāja instruk..2000,98 lpp.

29. Himmelfarb J., *et al.* Increased Reticulated Platelets in Dialysis Patiens, *Kidney Int*., 1997 – 51 – p. 834–839.
30. Hirase Y., Makatsuka H., Kawai T., Horiguchi S., Ikeda M. Stable blood cell count after one-week storage at room temperature // *Bull Environ Contam Toxicol*. – 1992 – 49(4), – p. 8–504.
31. Hofbauere G. Laboratorijas analīzes. Padomdevējs pacientam. Rīga: JUMAVA – 2006. 21.lpp.
32. Horbett T.A., Weathersby P.K. Adsorbtion of proteins from plasma to a series of hydrophilic hydrophobic copolymers. I. Analysis with the *in situ* radioiodination technique. // *J.Biomed. Mater. Res.* – 1981 –Vol. 15 – p. 403–423.
33. Hsin-Kai Chen, Gabi Nindi Waite, Penney L. Miller, Ellen F. Hughes, Lee R. Waite. Monitoring temperature and light exposure of biosamples exposed to ultraviolet and low energy radiation. // *Biomedical Sciences Instrumentation*, – 2007 – Nr. 43 – p. 312–317.
34. Jacqueline H., Bernadette F. Clinical hematology atlas. – Indianapolis, 2004. – p. 256.
35. Joo, L., Ong, D.L. David, L. Carnes & Kazuhisa Bessho. Evaluation of titanium plasma-sprayed and plasma-sprayed hydroxyapatite implants in vivo. Biomaterials, August 2004, Vol.25, Issue 19, pp. 4601-4606.
36. Kafka M., Yermiahu T. // *Clin. Lab. Haematol*. – 1998 – Vol.20, №4 – p. 213–216.
37. Kailner A., Quality management in the medical laboratory: a comparison of draft standarts. // ELSEVIER., *Clinica Chimica Acta* – 1998 – 278 – 111–119.
38. Kishida A., Kato S., Ohmura K., Sugimura K., *et.al.* Evaluation of biological responses to polymeric biomaterial br RT-PCR analysis. 1. Study of IL-1 beta mRNA expression. // *Biomaterials*. – 1996. – 17, No 13. – p.1301–1305.
39. Klīniska medicīna. Prof.Aivara Lejnieka redakcija. Medicīnas apgads. 2010. 942 lpp.
40. Kononen M., Hornia M., Kivilahti J., Hautaniemi J., *et al.* Effect of surface processing on the attachment, orientation, and proliferation of human gingival fibroblasts on titanium. // *J. Biomed. Mater. Res*. – 1992. – 26, No 10. – p. 1325–1341.
41. LaFayette N., *et al*. An *in vitro* method for assessing biomaterial-asociated platelet activation. *ASAIO J*., 2007 – 53 – p. 159–162.
42. Lee S.S. et al., Strain Handening of Red Blood by Accumulated Cyclic Suprphysiological Stress , Artif. Organs, 31, 2007. p. 80-86.
43. Lejniece S. Klīniskā hematoloģija. Rīga: Nacionālais medicīnas apgāds, 2005. – 324 lpp.
44. Levy R.J., Shoen F.J., Howard S.L., Oshry L. Mechanism of glutaraldehyde porcine aortic valve calcification // *Trans. Biomater*., 1983. – Vol. 6. – p. 98–103.
45. A.Leice, Y.Dekhtyar, N.Britzina, V.Arhipovich and L.Arabere. Human Blood Cells Affected by Hydroxyapatite Coated Titanium. 14th Nordic-Baltic conference on Biomedical Engineering and Medical Physics. Abstracts. June 16 – 20, 2008, p.147. Starptautiskas konferences grāmatā. Rīga, Latvija, Jūnijā 16 – 20, 2008.

46. Li D.J., Ohsaki K., Ili K., Cui P.C. *et al.* Thickness of fibrous capsules after implantation of hydroxyapatites in subcutaneous tissue in rats. // *J. Biomed. Mater. Res.* – 1999. – Vol. 45, No 4, – p. 322–326.
47. Lian I.B., Bernhard W., Levy R.J., Szycher M. Blood material interactions // Devices and technology branch contractors meeting proceedings, 1981. – p. 177.
48. Lian I.B., Levy R.J., Bernhard W., Szycher M. LVAD mineralization and j-carboxyglutamic acid containing proteins in normal and pathologically mineralized tissues // *Trans. Amer. Soc. Artif. Intern. Organs*, 1981. – Vol. 27. – p. 683–690.
49. Long J., De Santis S., Shors E. *Et al.* Thrombogenicity and the interaction of proteins, platelet and white cells. // Biomaterials, Med. Dev., Artif. Organs. – 1983. – Vol. 11. – p. 633–72.
50. Mahmodi M., Hajizadeh M., Rashidinejad H., Asadikaram G. Survey of changes in complete blood count and red cell indices of whole blood incubated *in vitro* at different temperatures up to 48 hours // *J Ayub Med Coll Abbottabad*. –2006 – 18(1).
51. Maloney W.J., Smith R.L., Castro F., Schurman D.J. Fibroblast response to metallic debris *in vitro*. // *J. Bone Joint. Surg*. – 1993. – 75-A, No 6. – p. 835–844.
52. Marois Y., Guidon R., Roy R., Vidovsky T., *et al*. Selecting valid *in vitro* biocompatibility tests that predict the in vivo healing response of synthetic vascular prostheses. // *Biomaterials*. – 1996. – 17, No 19. – p. 1835–1842.
53. Medicīnas laboratorijas. Ipašas prasības kvalitātei un kompetencei.”LVS EN ISO 15189”, sadaļa C.9, 2007.
54. Mueller X., *et al*. Hemolysis and hematology profile during perfusion; Inter-species comparison. // *Int. J. Artif. Organs,* – 2001 – 24 – p. 89–94.
55. Negroiu Gabriela , Piticescu Roxana M. , Chitanu Gabrielle C., Mihailescu Ion N., Zdrentu Livia and Miroiu Marimona. Biocompatibility evaluation of a novel hydroxyapatite-polymer coating for medical implants (in vitro tests) Journal of Materials Science , LLC 2007/10 / 007-3300-6
56. Nishimura Ichiro et al. Discrete deposition of hydroxyapatite nanoparticles on a titanium implant with predisposing substrate microtopography accelerated osseointegration. 2007. Nanotechnology 18 245101 (9. pp).
57. Ozoliņš D. Preanalītiskā posma kontrole – attīstības tendences // Medicine – 2007 – Novembris, 8.–10. lpp.
58. Pakr JY. Davies JE. Red blood cell and platelet interactions with titanium implant surfaces. Clin Oral Implants Res. 2000 Dec;11(6):530-9.
59. Patient – centred care – preanalytical factors demand attention // *Scand J Clin Lab Invest* – 2007 Nr.67, 836.–847. lpp.
60. Paul J., *et al*. Influence of Uremia on Polymorphonuclear Leukocytes Oxidative Metabolism in Endstage Renal Disease and Dialysed Patients. // *Nephron*, 1991 – 57 – p. 428–432.

61. Pinkowski R. Лейкоциты. Автоматический анализ, трудности, разногласия. – Lodz: Artdruk, 2000. c. 155.
62. Pizzoferrato A., Vespucci A., Capetti G., Stea S. Biocompatibility testing of prosthetic implant materials by cell cultures. // *Biomaterials*. – 1985. – 6. – p.346–351.
63. Praktiskā alergoloģija. Vernera Lozovska redakcijā. LU Akadēmiskais apgāds, 2009. – 623 lpp.
64. Pūga I. Dažādu faktoru ietekme uz asins analīžu rezultātiem. // *Jums, Kolēģi*. – 1996. – Nr.8, – 36. lpp.
65. Rodriguez-Lorenzo L.M., Vallet-Reg M. and Ferreira J.M.F. "Fabrication of hydroxyapatite bodies by uniaxial pressing from a precipitated powder". 2001. Biomaterials, 22, 583-588.
66. Schuler M., Gethin Rh. Owen, Douglas W. Hamilton, Michael de Wild, Marcus Textor. Biomimetic modification of titanium dental implant model surfaces using the RGDSP-peptide sequence: A cell morphology study. Biomaterials Volume 27, issue 21, July 2006. pp. 4003 – 4015.
67. Sinkeviča D. Laboratorijas Testu Interpretācijas Rokasgrāmata Ārstiem – Nacionālais Medicīnas Serviss – Laboratorija – 2008. – 52.–87.lpp.
68. Stenner E., Piccinini C., Biasioli B., Bussani A., Delbello G. Red cell and core temperature in spelunking. // *International Journal of Sports Medicine*, – 2007 – vol.28, Nr.9,– p.727–731.
69. Szycher M. Thrombosis, hemostasis and thrombolysis at prosthetic interface // Biocompatible polymers, metals and composites / Ed. Szycher M., Techom. Publ. Co. Inc., 1983. – p. 1–33.
70. Thomson L.A., Law F.C., James K.H., Matthew C.A, *et al*. Biocompatibility of particulate polymethylmethacrylate bone cements: a comparative study *in vitro* and *in vivo.* // *Biomaterials.* – 1992. – 13., No 12. – p. 811–818.
71. Tjuļkins F. Bioimplantu un asins šūnu morfoloģijas un mehānisko ipašību pētījums ar atomspēka mikroskopu.// Rīga, RTU, bakalaura darbs.-2009. – 35.lpp.
72. Veliks V. Magnētiskais lauks un tā ietekme uz organismu. Doctus. 2005.№ 8,44.–47.lpp.
73. Viayanand K., Deepak K. Pattanayak, T.R. Rama Mohan and R.Banerjee. Interpreting Blood-Biomaterial Interactions from Surface Free energy and work of Adhesion. In: Trends Biomater. Artif. Organs. 2005. Vol 18 (2), January
74. Vienken J. The need for a systems approach in biomaterials testing. // MDT — September 2007 — Germany. http://www.devicelink.com/mdt/archive/07/09/004.html
75. Vijayanand, K., Deepak, K., Pattanayak, K., Rama Mohan T., & R.Banerjee, R.,Interpreting Blood-Biomaterial Interactions from Surface Free Energy and Work of Adhesion. Trends Biomater. Artif. Organs, Vol. 18 (2), January 2005. pp. 73-83.
76. Vinķele R. Medicīnas svešvārdu vārdnīca. — Rīga: Avots, 2007, 959 lpp.
77. Vogelaar S.A., Posthuma D., Boomsma D., Kluft C. Blood sample stability at room temperature for counting red and white blood cells and platelets. // *Vascular Pharmacology* – 2002 – Nr.39 – p. 123–125.

78. Wooley P.H., Nasser S., Fitzgerald R.H. jr.The immune response to implant materials in humans. // *Clin. Orthopaed.* – 1996. – 326. – p.63–70.
79. Zaharāne S. Preanalītiskie faktori // Latvijas Ārsts. – 1995 – №6 – 40.–43. lpp.
80. Балаба Т.Я., Красовская Г.П., Слуцкий Л.И., Торбенко В.П. Некоторие данные о биохимических механизмах репаративного остеогенеза.*Труды II всес. сьезда травматологов-ортопедов*. Рига, 1969, М., 1970. с. 140–144.
81. Биология. Под ред. В.Нярычина, М.: Медицина, 1985. – с.560 (397).
82. Бобро Л.И. Фибробласты и их значение в тканевых реакциях. // *Арх. Патол.* – 1990. – 52, № 12, с. 65–68.
83. Бордюшков Ю., Горошинская И., Франциянц Е., Ткачева Г., Горло Е., Нескубина И. Структурно-функциональные изменения мембран лимфоцитов и эритроцитов под воздействием переменного магнитного поля. // *Вопросы медицинской химии*, – 2000 – Nr.3.
84. Борисов Ю., Спиридонов В., Суглобова Е. Резистентность эритроцитарных мембран: механизмы, тесты, оценка // *Клиническая лабораторная диагностика*. – 2007 – №12, 36–40 с.
85. Вохминцев П.А., Сайфиев Р.Р., Фролова О.В. Экзогенная регуляция деформабильности эритроцитов. // *Успехи современного естествознания* – 2004 – № 2 – с. 46.
86. Друх В., Фархутдинов Р., Загидуллин Ш. Метод изучения хемилюминесценции лейкоцитов цельной крови // *Клиническая лабораторная диагностика*. – 2004 – №12 – 41–43 с.
87. Елемесов Р.Е., Акоев В.Р., Ким Ю.А. Влияние электромагнитного излучения на гемолиз эритроцитов, истощенных по АТФ. // II съезд биофизиков России. Тезисы. – Москва, 1999.
88. Жербин Е., Чухловин А. Радиационная гематология. – Москва: Медицина, 1989, – 176 с.
89. Зинчук В.В. Деформируемость эритроцитов: физиологические аспекты. // *Успехи физиологических наук*. – 2001. – Т. 32, № 3. – с. 66–78.
90. Каманина Н.В. Отличие (и взаимосвязь) эффекта ориентирования эритроцитов в нематической жидкокристаллической среде от электрический вибраций Fröhlich'a. // *Письма в ЖТФ* – 1997 – том 23, Nr.23.
91. Клинико–диагностическое значение лабораторных показателей / Долгов В, Морозова В., Марцишевская Р. и др. – М, – 1995. – 215 с.
92. Козинец Г. ред., Макаров В. Исследование системы крови в клинической практике. – Москва: Медицина, 1997.– 480 с.
93. Козинец Г., Погорелов В., Дягилева О., Наумова И. Кровь. – Москва: Медицина, 2006.– XXI – 251 с.

94. Козинец Г.И., Каюмова Д.Ф., Погорелов В.М. Клетки переферической крови и экологические факторы внешней среды. // Клиническая и лабораторная диагностика. – 1993 – .No.1 – с. 14–20.
95. Козинец Г.И., Погорелов В.М. и др. Клетки крови. Современные технологии их анализа. – М: Триада – Фарм, 2002 – с.534.
96. Козинец Г.И., Шишкапова З.Г., Сарычева Т.Г., и др. Клетки крови и костного мозга. Цветной атлас. – М.: МИА, 2004. – с. 202.
97. Козинец Т.И. Анализы крови и мочи. Клинического значение. – М.: МИА, 2006. – с. 99.
98. Кузник Б., Максимова О. Общая гематология. – Чита: Феникс, 2007. – 573 с.
99. Лаврентьев Б.Л., Моисеев Ю.В., Русецкий А.Н., Садов Б.Д. Кинетика адсорбции гамма-глобулина на стеклянной поверхности, регистрируемая методом флюоресценции при полном внутреннем отражении. // Лаб. Дело. – 1982. – № 10. с. 612–612.
100. Лакин К.М., Макаров В.А., Новикова Н.В. Пути лекарственной регуляции функции гемостаза // Актуальные вопросы исследования системы гемостаза в клинической практике. Материалы симпозиума. Москва–Вена. – 1985, 27–28 марта – с. 53–57.
101. Лаксина О.В., Пестова М.Б., Немец Е.А. Влияние чистоты и природы полиэфирной составляющей на гемосовместимые свойства полиуретанов // *Проблемы трансплантологии и искусственных органов*. – М., 1981 – с. 150–152.
102. Мансурова Л.А., Скорнякова А.Б., Казимировская В.Б., Трофимов В.В., Слуцкий Л.И., Воронков М.Г. Изучение биосовместимых свойств имплатируемых биоматериалов. // *Докл. АН*. – 1996. – 350, No.3. – с. 408–410.
103. Мансурова Л.А., Скорнякова А.Б., Севастьянова Н.А., Казимировская В.Б., Слуцкий Л.И., Жданкович Е.Л., Абзаева К.А. Иследование биосовместимости новых полимерных материалов. // *Хим. Фарм. журнал*. – 1991. – 25, No 9. с.19–20.
104. Мизун Ю.Г.,Хаснулин В.И. Наше здоровье и магнитные бури.М.Знание,1991.190 с.
105. Мошкин А.В., Долгов В.В. Обеспечение качества в клинической лабораторной диагностике: практ. рук. – М: Медиздат, 2004. – 216 с.
106. Павиченко О., Калиман П. Влияние хлорида кадмия на развитие оксидативного стресса в легких крыс. Харьковский национальный университет, Украина, 2003.
107. Позняк С.Н., Позняк Н.И., Барковский Е.В., Лобанок Е.С., Лаврукевич Т.В. Ультразвук факоэмульсификатора и состояние клеточных мембран. БГМУ, Информация. МЖ – 2001.3 (21), 2001.
108. Покровский В., Коротько Г. Физиология человека. Москва: Медицина, 2007. с. 655.
109. Поливода Б.И., Конев В.В., Попов Г.А. Биофизические аспекты радиационного поражения мембран. М.: Энергоатомиздат, 1990. 153 с.
110. Рогожников Г.И., Немировский М.Б. Сплавы титана в ортопедической стоматологии. Пермское книжное издательство, 1991.с.6-27.

111. Сайфиев Р.Р. Исследование деформируемости эритроцитов млекопитающих с использованием усовершенствованного эктацитометра. Автореферат диссертации на соискание ученой степени кандидата биологических наук. Тюмень. 2002. 24 с.
112. Севастьянов В.И., Лаксина О.В., Новикова С.П., Розанова И.Б., Цейтлина Е.А., Шальнев Б.И. Современные гемосовместимые материалы для сердечно-сосудистой хирургии. Медицина и здравоохранение. Выпуск 2. Москва, 1987. с. 72.
113. Селезнев А., Ненашев А., Кондурцев В. Методы определения механической резистентности эритроцитов и характеризующие ее показатели у здоровых людей. *Клиническая лабораторная диагностика*. 2002. №5. 41–43 с.
114. Слуцкий Л., Ветра Я. Биологические вопросы биоматериаловедения. Рига: Латвийская Медицинская Академия, 2001. 150 с.
115. Трофимов В.В., Клименов В.А., Казимировская В.Б., Мансурова Л.А. Иследование биологической совместимости гидроксиапатита. *Стоматология*. 1996. 75, No 5. – с. 20–22.
116. Хепч Л., Джонс D., Биоматериалы, искуственные органы и инжиниринг тканей. Мир биологии и медицини, М.: Техносфера, 2007. с. 303.
117. Шмаров Д.А., Козинец Т.И. лабораторно-клиническое значение проточно-цитометрического анализа крови. М.: МИА, 2002. с.127.
118. Шмидт Ю. Влияние антистрессового препарата „Аминовит” на морфологию надпочечных желез крыс и морфо-цитохимические показатели крови телят при транспортном стрессе. – Автореферат, Новосибирск, 2003.
119. Экологические факторы кроветворения. Российский научный симпозиума.М., 1992.
120. Яструбинецкая О., Попова О., Сарычева Т., Тенцова И., Козинец Г. Определение электрофоретической подвижности эритроцитов периферической крови здоровых людей с использованием в качестве консерванта этилен-2-амино-4-хлоруксусной кислоты (ЭДТА). *Клиническая лабораторная диагностика*. 2009. №1. 22, 35.36 с.