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Digital Processing Techniques Influence on Resolution of Acousto-Optical Blood Typing Method

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The various digital techniques of photo images processing influence upon the resolution of the acousto-optical method (AOM) for instrumental blood typing according to the AB0 system has been studied. Experiments with blood samples were carried out using monoclonal antibodies (tsoliclones) of anti-A and anti-B types, as well as without them („clean" sample) when the mixture was exposing simultaneously by a standing ultrasonic wave. The experiment was recorded by a digital camera. Different variants of statistical calculations have been proposed to process the photo images of the recorded phenomena, the results were compared with the traditional photometric approach. It is shown that the statistical processing of experimental results gives a higher resolution in blood group determination and, consequently, a higher reliability of its typing. A comparison of different variants for statistical photo frames processing was carried out to study the possibility to increase the resolution of AOM in blood typing.

Keywords: acousto-optical blood typing method, monoclonal antibodies, digital processing techniques, blood typing, high resolution.

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1. Introduction

Blood typing as per AB0 system is one of the most common tests of laboratory diagnostics. From 150 to 200 mln. clinical analyses on blood typing are annually carried out in USA alone [1]. Comparing the population size of USA and Russia, it can be expected, that the amount of tests, carried out in Russia annually for blood typing, is nearing to 100 mln. Obviously, such extremely high frequency of such tests carrying out requires creation of special equipment, automated devices for instrumental blood typing [2–9].

One of the most important characteristics of such devices is resolution R. Authors of various works define this parameter differently. For instance, in the works [4,5] the resolution R is defined as a degree of slope difference of spectral curves for positive and negative reactions respectively in a wave lengths range from 650 to 1000 nm. It should be explain, that agglutination reaction is considered positive, if serum (monoclonal antibodies) is immunologically appropriate to the examined blood type — erythrocytic agglutinates are formed; otherwise the agglutination reaction is considered negative (agglutinates are not formed).

The works [6–8] are dedicated to improvement of this spectrophotometric method of blood typing. It should be noted, that in [4,5] the limit value of „resolution" was $R_{\text{limit}} = 17$, i.e., if at experiment with this blood sample and serum (monoclonal substance) $R < R_{\text{limit}}$, the agglutination reaction is considered negative. And vice versa — at $R > R_{\text{limit}}$ the reaction is positive. As a result, such analysis allows the typing of this blood sample. However, the obtained values of resolution R are not very high — increase of device resolution remains the vital task, since it increases reliability of blood typing. It should be noted, that error in blood sample typing should be completely excluded. At insufficient device resolution and due to high variation of blood samples the device may not define the analyzed sample type, but error in blood typing is unacceptable. In [9,10] the resolution is proposed to define as relation of optical signals, for instance, power of probing light streams $R = P_+/P_-$, corresponding to positive and negative agglutination reactions.

Among publications on devices for instrumental blood sample typing the works [11, 12] should be noted; the works [13,14] are dedicated to development of approaches to such systems design based on flow cytometry.

One of the techniques of resolution increase is application of AOM for human blood typing. AOM consists in the following. At exposure of standing ultrasonic wave to the examined mixture of „blood solution + hemagglutinating serum and monoclonal antibodies (tsoliclones)" in case of positive reaction of erythrocytes agglutination in standing-wave nodes the large agglutinates are formed, which quickly sedimentate at ultrasound (US) turning off. At the same time at negative reaction the ultrasound turning off results in destruction of RBC (red blood cell) of aggregates, while small aggregates and free erythrocytes slowly sedimentate. In [15] it is experimentally showed, that at ultrasonic exposure the agglutinates sedimentation rate at positive reaction exceeds the aggregates sedimentation rate at negative reaction by more than an order. It means, that at some point of time at positive reaction the examined

medium becomes transparent almost completely, while at negative reaction it remains turbid. The corresponding optical measurements show, if the agglutination reaction occurred for presented mixture of „the examined blood solution + haemagglutinating agent“This principle is used for blood typing of the analyzed blood sample.

We presented this method for the first time in analog form in [9] and described it in detail in [16]. In [17] it was proposed for the first time to use a digital camera for AOM of blood typing, therefore to use the digital processing of the experimental results. Then the various options of AOM of blood typing were examined based on various physical principles using digital camera, e.g. methods of flow cytometry [18] and digital microscopy [19].

It is important to note, that AOM particularly allows to obtain the high resolution, therefore the high certainty of blood typing. Thus, if in the first works of this area [9,10] the resolution varied from single units to several dozens, the development of AOM of blood typing (application of various optical methods of agglutination reaction registration) increased this parameters to hundreds and thousands [15]. This allowed to increase certainty of blood typing and, also, to define the typing in case of samples with low agglutination.

In the works [20,21] the blood typing is performed using hemagglutinating serums based on the following techniques of photo images processing: photometric approach; static technique, including analysis of dispersion of pixels distribution for certain observation zone by their brightness, count of number of photo image pixels with the set brightness, calculation of summary brightness of photo image pixels. Comparison of the obtained resolution values for AOM of blood typing was performed for the above mentioned techniques of photo image processing.

However, recently the tsoliclones (anti-*A* and anti-*B*) found their use in transfusion practice instead of traditional hemagglutinating serums. Of course, the significant differences of their agglutination activity should be expected due to different nature of these agents. Besides, since hemagglutinating serums, as well as tsoliclones, are colored, that also increases the difference in determination of erythrocytes agglutination degree through optical technique using these agents [22]. It is shown, that hemagglutinating serums color not just impacts the technique resolution, but can result in errors in blood typing - especially at low agglutination ability of samples.

Therefore our work has the following objectives:

1) experimental comparison of influence of various techniques of photo images processing on resolution of AOM of blood typing under conditions of application of tsoliclones of anti-*A* and anti-*B* types as agents;

2) search of ways of photo images processing techniques development to increase AOM of blood typing resolution.

2. Materials and experimental technique

Donor blood solutions were used as study objects. Three samples were made for each examined blood sample. The first two: ?whole blood + physiological saline + tsoliclone“(anti-*A* and anti-*B*). At the same time the relation of volumes of „whole blood−tsoliclone“ was 1 : 10. The third sample: „whole blood + physiological saline“ does not contain tsoliclone („pure“ sample) This sample was used as a „reference“ — there is no erythrocyte agglutination reaction here in principle due to lack of tsoliclones in the sample. To an extent this sample is an equivalent of the case of erythrocytes agglutination negative reaction. Introduction of such test is determined by desire to improve reliability of blood typing, that is one of the principal differences of this work from [15,20] and others. Indeed, for blood typing of the examined blood sample with three experiments carrying out with US exposure to the samples, only one of them is a priori known as the one without agglutination reaction. Then the results of experiments with tsoliclones participation should be compared with exactly this sample.

Cuvettes with dimensions of $5 \times 18 \times 32$ mm were used for the experiment, the sample volume in the cuvet was $2500\,\mu$l. Blood percentage in all examined samples was 2% of total volume. Relation of the components volumes was the following.

- For samples with tsoliclone „whole blood: tsoliclone: physiological saline“$= 1 : 10 : 36 = 2 : 20 : 78\% =$ $= 50 : 500 : 1950\,\mu$l.
- For „pure“ sample „whole blood: physiological saline“$= 1 : 49 = 2 : 98\% = 50 : 2450\,\mu$l.

Further on we will call the samples of the three mentioned types as the bio-objects of the study.

Experiments were performed in two stages: samples preparation, and then registration (video recording) of erythrocytes and their associates (aggregates — in case of negative reaction, agglutinates — in case of positive reaction) sedimentation. Whole blood and physiological saline were mixed in the corresponding proportions during samples preparation in cuvettes. In case of samples with tsoliclone, due to high agglutination activity this component was added right before the start of the registration process, when the examined sample was already at the experimental setup (fig. 1). Video recording started immediately after the whole sample mixing.

10 s after the registration start for 50 s the sample was subject to US exposure. Time of registration of erythrocytes and their associates sedimentation, and, consequently, the time of determination of presence or lack of agglutination reaction (video duration) was 2 min. This time was sufficient for, in cases of positive agglutination reaction, the process of agglutinates sedimentation to be subject to „saturation“.

3. Experimental setup

Experimental setup is presented in fig. 1, *a*. Cuvet with the examined mixture was located on a piezoelectric converter, US wave was oriented upwards. Generator GZ-112/1 with amplifier was used for piezoceramic converter excitation, while its output voltage was controlled

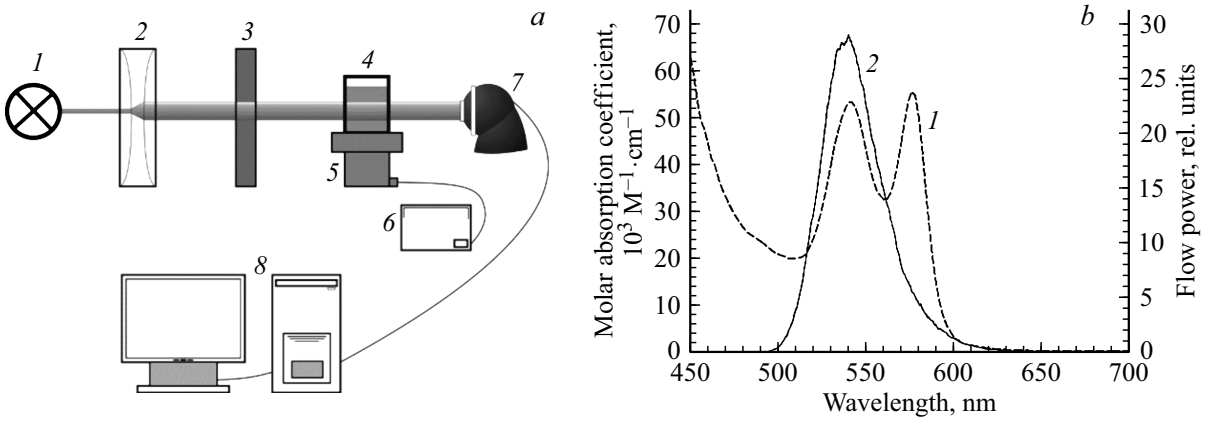

Figure 1. (*a*) Scheme of setup for registration of erythrocytes agglutination reaction: *1* — LED, *2* — condenser, *3* — neutral light filter, *4* — cuvet, *5* — ultrasonic converter, *6* — ultrasonic generator, *7* — eight-bit digital photo camera, *8* — computer. (*b*) Spectra: *1* — hemoglobin absorption (left ordinate axis), *2* — LED radiation (right ordinate axis).

with oscillograph S1-79. The generator was set resonantly relating to the converter $\nu = 2.25$ MHz, while its output voltage, supplied to piezoceramics, did not exceed 15 V, thus providing the ultrasonic exposure to erythrocytes without their hemolysis.

Bio-object was probed with collimated radiation of LED of LXHL-G1S type with maximum radiation flow power at a wave length of 540 nm (fig. 1, *a*), spectrum of which corresponded to hemoglobin absorption spectrum in the green section (fig. 1, *b*). LED power mode: voltage 3 V, current strength 0.3 A. Selection of the probing light radiation spectrum was determined by necessity of photo image contrast increase, that contributes to the blood sedimentation registration accuracy improvement.

Light beam, transmitted through the examined solution, entered the polychrome web camera Logitect-QuickCam, that registered the process of RBC associates sedimentation with frequency of 15 frames per second. Videos were broken down into photo frames with interval of 1 s. The observed digital images were broken down to RGB (red, green and blue) components, while only the green one was analyzed. That choice is associated with hemoglobin light absorption in the green section, and, of course, with LED probing radiation spectrum.

4. Experimental results

The results of experiments, subject to the further computer processing, are digital photo images of the bio-object sedimentation process (fig. 2).

Photo images in fig. 2 correspond to the following moments of time: *a, d, g* — right before US turning on; *b, e, h* — immediately after US turning off; *c, f, i* — 1 min after US turning off. According to fig. 2, the images of $(e)-(h)$ and $(f)-(i)$ are similar pair-wise. Pair of $(e)-(h)$ demonstrates the formation of erythrocytic aggregates, the second $(f)-(i)$ — RBC aggregates dividing into separate free erythrocytes or smaller aggregates. In the second case the suspension remains transparent, and images (f) —(i) are almost identical. Situation changes in case of positive reaction: with US turned off the formed large agglutinates (fig. 2, *b*) quickly sedimentate, while the medium significantly brightens up (fig. 2, *c*). Thus, using the value of the suspension transmission coefficient in the cuvet (photometry) or its photo image brightness, it is possible to identify, for which monoclonal antibodies (anti-*A* or anti-*B*) the positive reaction occurs. Based on that the type of the examined blood sample can be defined according to table 1.

Table 1. Blood typing of the blood sample by agglutination reaction type

Tsoliclone		Type identity of a sample
anti-*A*	anti-*B*	
Agglutination reaction type		
—	—	0(I)
+	—	A(II)
—	+	B(III)
+	+	AB(IV)

Note: Indices „—“ and “+“ correspond to positive and negative agglutination reaction respectively.

5. Techniques of photo frames digital processing

The observed experimental photo images, like on fig. 2 were processed using several computer techniques.

1. Photometry. 2. Static techniques: • dispersion analysis of photo image pixels distribution by brightness for positive and negative agglutination reactions;

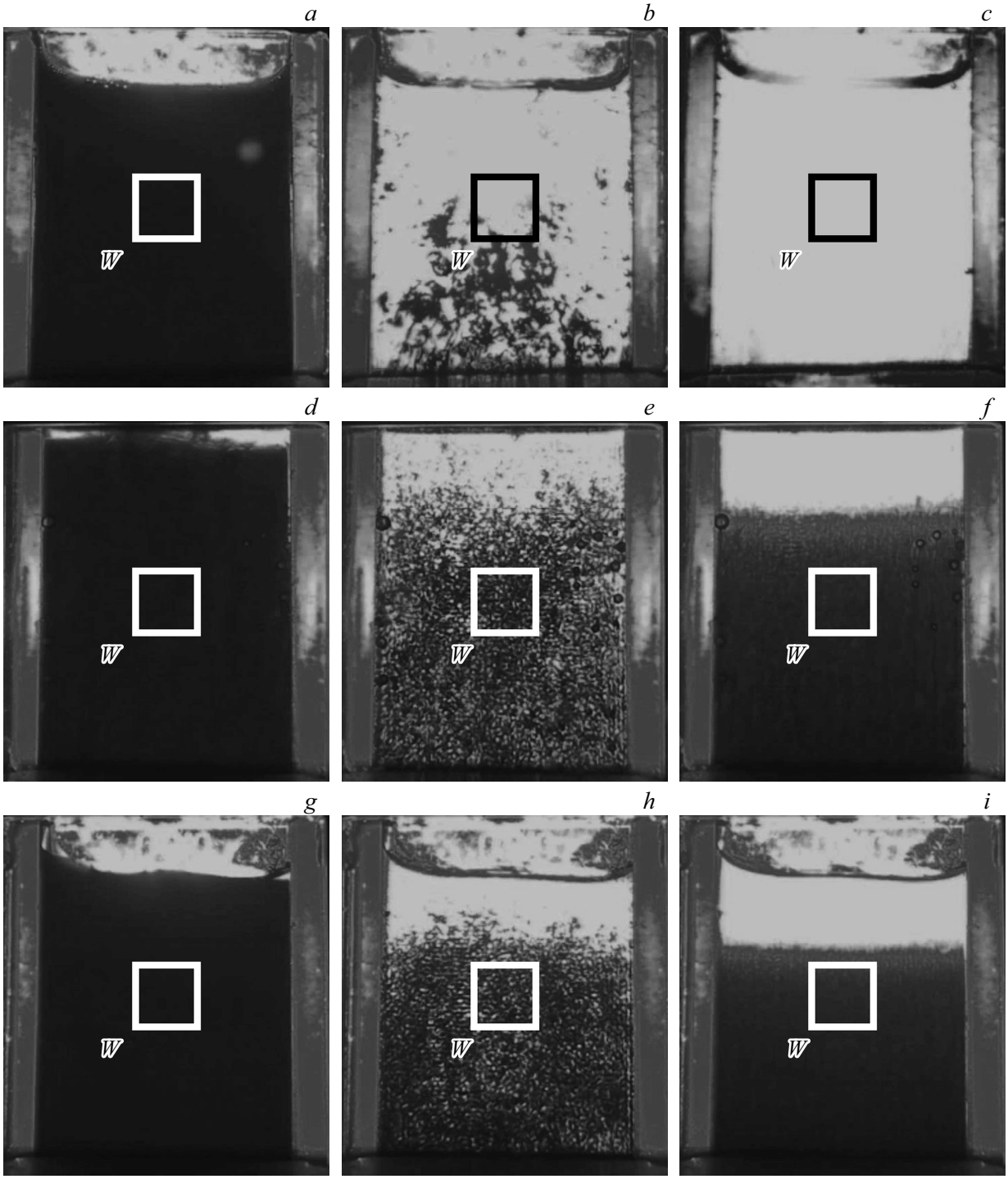

Figure 2. Examples of the examined digital photo images: *a, b, c* — positive agglutination reaction; *d, e, f* — negative agglutination reaction; *g, h, i* — „pure“ sample. *W* — area of photo images computer processing (100×100 pix).

• calculation of distribution of pixels number by brightness for positive and negative agglutination reactions using brightness limit value;

• calculation of summary brightness of photo image pixels for positive and negative agglutination reactions;

• determination of average pixels distribution density by brightness for positive and negative agglutination reactions.

For each examined processing technique the analyzed image was subject to the following operations:

1. Allocation of zone W on a photo image (fig. 2). 2. Image conversion in zone *W* into two-dimensional numeric array for each RGB component.

3. Allocation and processing of the „green“ component only.

As a result, for each examined blood sample and every processing technique after the experiment completion two parameters were obtained, that demonstrate, for instance, brightness exceeding degree B (B — brightness) of the examined sample with tsoliclone compared to the „pure“ sample. Thus, for instance, if $R_a = B_a/B_0 \sim 1$, it means, that sample brightness B_a with tsoliclone of anti-A type after the experiment completion is close to brightness of the „pure“ sample B_0 ($B_a \sim B_0$) — there is no corresponding immune reaction (reaction is negative, $B_a = B_-$).

In this case the value of R_a can be considered as just a „relative resolution“ relating to tsoliclones of anti-A type. If, for instance, $R_b = B_b/B_0 \gg 1$, i.e. $B_b > B_0$ (B_b — brightness of sample with tsoliclone of anti-B type), then the agglutination reaction occurred, the reaction is positive ($B_b = B_+$). In this case the examined blood corresponds to type B (group III), R_b — „relative resolution“ relating to tsoliclones of anti-B type. When changing the relations for R_a and R_b to the opposite ones, for instance, $R_b = B_b/B_0 \sim 1$, but $R_a = B_a/B_0 \gg 1$, blood type A (group II). Analysis of the relations between the values of „relative resolutions“ R_a and R_b allows to define the type of the examined blood samples.

It is important to note, that unlike many works on the examined topic, published earlier, here we propose for the first time to use the „pure“ sample — equivalent of a priori negative agglutination reaction. Its purpose not only in improvement of blood typing certainty, but also in provision of this task solution reliability for blood samples with low agglutination, as well as for blood samples of type 0(I) and AB(IV). The problem with the latter samples is in the fact, that these samples react with both tsoliclone types the similar way: blood sample 0(I) in both cases (anti-A and anti-B) reacts as negative, while for AB(IV) in both cases the positive agglutination reaction is observed (table 1). In these cases the application of the „pure“ sample is preferable, since it is an equivalent of the negative agglutination reaction a priori.

It should be noted, that for all image processing techniques the resolution calculation algorithm was common. Thus, after US exposure turning off, 30 s were allocated for the sample incubation (time intended for sedimentation of agglutinates, free erythrocytes and their aggregates), then the processing was implemented — also for 30 s.

Values, that are presented in paragraphs of sect. 5, are related to the experimental results processing with various techniques, i.e. digital photo images, observed in sect. 4.

5.1. Photometric technique of photo images processing

Similar to [20] the photometric technique of photo images processing is defined as determination of the average pixels brightness $\bar{B}$ over the section of W (fig. 2) and its processing time (fig. 3). The latter should be explained: as an example, the curves of the average pixels brightness dependence on observation time for three blood samples of a single certain blood specimen are built in fig. 3. Two zones are specified in this figure: samples incubation zone — from 60 to 90 s (incubation) and their processing zone — from 90 to 120 s (processing).

As seen in fig. 3, for photometric technique of the results processing the values of the „relative resolutions“ are $R_b = B_b/B_0 \sim 1.43$ and $R_a = B_a/B_0 \sim 0.86$. Therefore $R_b > R_a$ — suspension in the cuvet was brightened up with tsoliclone of anti-B type more, than with anti-A. It means, that this blood sample, as per table 1, belongs to group B(III). However, it should be noted, that integrally the resolution is just $R_{\text{ph}} = R_+/R_- = R_b/R_a \sim 1.7$ (index ph of the„relative resolution“ R_{ph} means photometry). Almost the same values of Rph were also characteristic for other examined blood samples: actually the maximum value of R_{ph} for 34 samples was not more than 3. In general, it is wrong to think, that the photometric technique of AOM of study results processing has sufficient resolution in blood group determination, especially for blood samples with low agglutination capability.

5.2. Static technique of photo images processing

The technique consists in static analysis of pixels distribution of zone W (fig. 2) by brightness. Examples of such experimental distributions are presented in fig. 4.

Distribution of pixels (fig. 4, *a*) for „pure“ sample (curve *1*) and negative agglutination reaction (curve *2*) correspond to the moment of time of 15 s after US exposure to the sample. Experiments demonstrated, that distribution for „pure“ sample is usually located on the right (higher brightness region) to the pixels distribution for negative reaction. This is caused by the fact, that tsoliclones of both types are specially colored, that defines the high light absorption of the probing radiation. At the same time the variability of various blood samples parameters sometimes results in reverse mutual arrangement of above mentioned distributions. However, the evaluations show that this does not really influence the values of „relative resolutions“ of R_a and R_b at analysis of negative agglutination reactions.

In case of positive reaction the sample translucence in the zone W starts almost immediately after ultrasound turning off (fig. 2 and 4): all pixels of zone W (10000) have almost the same brightness limit value (~ 200). Moreover, as was noticed in [14], sedimentation of agglutinates (therefore the sample translucence) starts even before US turns off. This indicates, that ultrasonic standing wave forms such large RBC agglutinates, that under gravity they sedimentate despite levitation. However, in case of negative reaction and „pure“ sample at the same moment of time a large amount of free erythrocytes and erythrocytic aggregates of small size are still in the zone W, absorbing the probing light stream.

It is important to note that for the further qualitative evaluations of „relative“ resolutions in sect. 5.2.2 and 5.2.3 the vertical lines ab and mn are drawn from points on axis „Brightness“, where 1) the minimum boundary brightness $B_{\text{bound}} \sim 98$ corresponds to the line ab, while number of

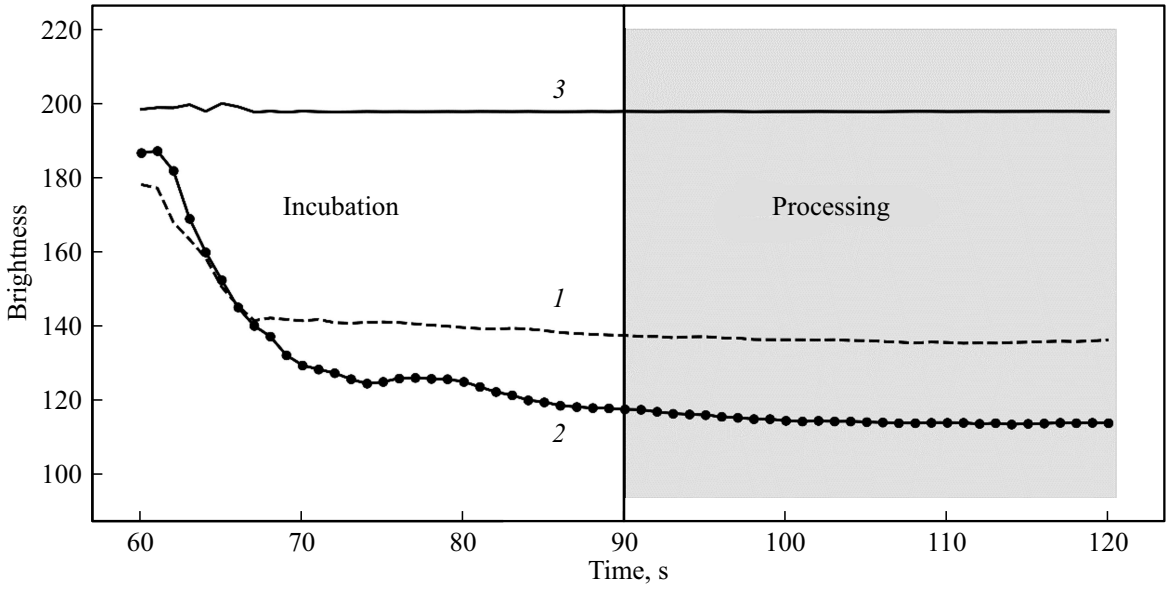

Figure 3. Dependences of average pixels brightness B in the zone W on observation time for various blood samples: *1* — „pure“ sample without tsoliclone; *2, 3* correspond to interaction of the examined blood sample with monoclonal antibodies of anti-*A* and anti-*B* respectively. This blood sample group identity — B(III).

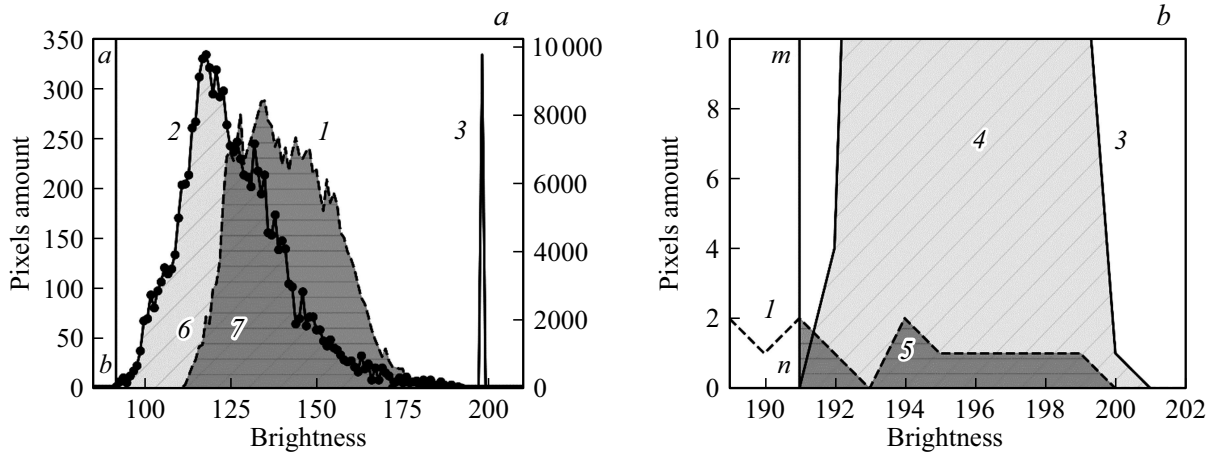

Figure 4. (*a*) Photo images pixels distribution by brightness: *1* — „pure“ sample, *2* — negative agglutination reaction, *3* — positive agglutination reaction. Curves *1, 2* — left ordinate axis, *3* — right ordinate axis, *ab* — boundary value line (Bbound) for pair of „pure sample“−negative reaction“, *6* and *7* — parts of areas of pixels distribution for negative reaction of RBC agglutination *S*- and „pure“ sample S_0 respectively if $B \geq B_{\text{bound}}$ for both cases. (*b*) Magnified image of the fragment of the same distribution (fig. 4, *a*) for „pure“ sample (*1*) and positive reaction (*3*), mn — boundary value line (B_{bound}) for pair of „pure sample−positive reaction“, *4* and *5* — parts of areas of pixels distribution for positive reaction of RBC agglutination S_+ of pure sample S_0' respectively if $B \geq B_{\text{bound}}$ for both cases.

pixels for negative reaction becomes zero, 2) $B_{\text{bound}} \sim 191$ corresponds to the line mn, where number of pixels for positive agglutination reaction also becomes zero. Of course, the above mentioned numbers are specified for distributions on fig. 4, *a* only. They are valid only for this photo frame, for other frames they are different and specially set.

And, finally, it should be noted, that pixels distribution for positive reaction is always on the right (higher brightness section) to the „pure“ sample and negative agglutination reaction case (fig. 4).

5.2.1. Dispersion analysis of photo image pixels distribution by brightness It is seen from fig. 4, *a*, that width of photo image pixels distribution by brightness for negative reaction (for the „pure“ blood sample also though) vastly differs from the same parameter for positive agglutination reaction. By approximating this distribution with normal, it can be assumed, that as a parameter, that allows to distinguish the positive agglutination reaction from the negative, the dispersion d can be chosen.

In this case $R_{d,a} = d_0/d_a$ and $R_{d,b} = d_0/d_b$ can be used as the „relative resolution“ (index d of the „relative resolu-

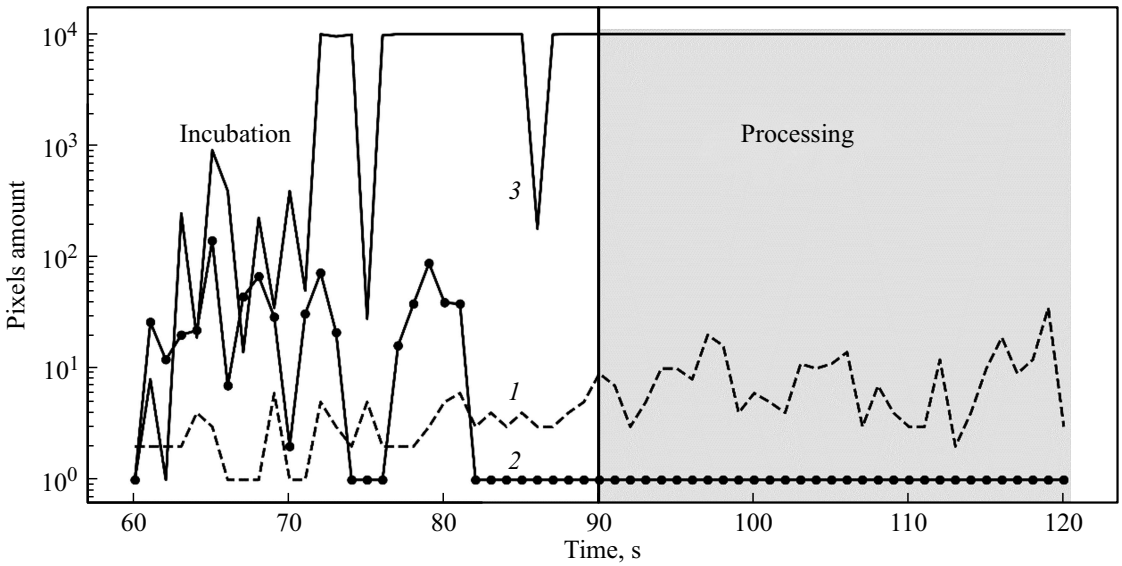

Figure 5. Dependences of N_a (*1*), N_b (*2*) and N_0 (*3*) on observation time.

tion" R_d means dispersion). Such approach at other equal conditions allows to hope for the higher value of resolution compared to photometric approach. Indeed, as shown in sect. 5.1, the value of R_{ph} is limited with several units, while dispersions for positive and negative agglutination reactions can differ more significantly (fig. 4, *a*). The results of this technique of the experiments processing are presented in table 3.

5.2.2. Calculation of pixels number with brightness, exceeding the specified boundary value To demonstrate this technique of photo images processing let?s examine only three synchronous photo frames of three videos, that correspond to positive, negative erythrocytes agglutination reactions and „pure" sample. The technique consists in the following.

For the examined blood sample we will perform the experiments with two antibody types (anti-*A* and anti-*B*) in sequence, while it is initially unknown which sample of two gives the positive agglutination reaction, and which — the negative. As was noted earlier, the position of pixels distribution for the negative reaction is close to the position of distribution for „pure" sample (fig. 4, *a*), pixels distribution for the positive reaction is significantly shifted to the right relating to the distribution for the negative reaction, and, consequentially, relating to the distribution for the „pure" sample. At the same time the „pure" sample is considered as a „reference". These two circumstances allow to define which of antibody types (which of two experiments) gives the positive reaction, and which — the negative. In general, this should allow to define the examined sample blood group.

However, for automated determination of the blood sample group identity, some numerical experimental data should be provided to computer, specifically the values of R_a and R_b. For that purpose let?s choose the positions of the lines an and mn (fig. 4) for the observed experimental distributions, as it is described in introduction to sect. 5.2.

Let's calculate separately the number of pixels for the negative reaction N_- and „pure" sample N_0 (fig. 4, *a*) under condition, that pixels brightness B satisfies $B \geq B_{\text{bound}}$, where B_{bound} is in point b. Obviously, the area *6* (negative reaction) and the area *7* („pure" sample) (fig. 4, *a*) under this condition symbolize the number of pixels N_- and N_0 respectively. It is also seen, that these areas are similar, therefore, $N_- \sim N_0$, i.e. $R_a = N_-/N_0 \sim 1$. Hereinafter the designation N — number of pixels. Let?s examine the similar parameter R_b for positive reaction (fig. 4, *b*).

In this case at the boundary of distributions for the positive agglutination reaction and for „pure" sample we choose some boundary value of brightness B_{bound} (point n). Here the areas *4* and *5* symbolize the number of pixels for the positive reaction N_+ and „pure" sample N_0 respectively. But now $N_+ \neq N_0$, moreover, $N_+ \gg N_0$. Then $R_b = N_+/N_0 \gg 1$. Quantitatively comparing R_a and R_b, the examined blood sample group can be determined.

Let's move from the processing of three certain photo frames for three samples of a single blood specimen to the analysis of three videos, registering the presence of lack of RBC agglutination. Kinetics of the process of the pixels number change is presented in fig. 5.

Processing of the analyzed synchronous photo frames of three videos (positive, negative agglutination reaction and „pure" sample) was performed in accordance with the above mentioned principles. It is important to note, that the boundary brightness values B_{bound} were determined for each examined moment of time (photo frame) and video type individually. Besides, the resolution R_N was determined as an observation time-averaged relation of pixels number with brightness, exceeding the boundary for the case with

Table 2. Criteria of blood typing of the blood samples

Parameter of processing of photo frames (section)	Maximum value „of realtive“ resolution for negative reaction, $(R_-)_{\max}$	Criterion, set in the computer program, (R_{cr})
1	2	3
D (5.2.1)	13	15
N (5.2.2)	64.9	100
S_N (5.2.3)	77.2	100
PDDB (5.2.4)	5.5	10

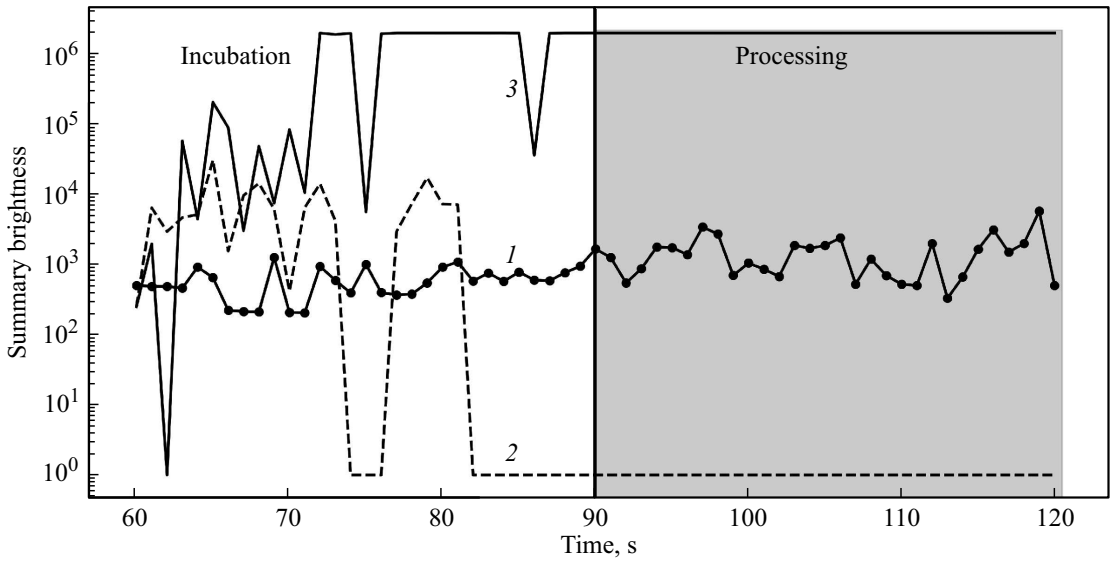

Figure 6. Dependences of summary pixels brightness on observation time: *1* — S_{N0}, *2* — S_{Na}, *3* — S_{Nb}.

tsoliclone ($\bar{N}_a$ or $\bar{N}_b$), and pixels number for the „pure“ sample ($\bar{N}_0$): $R_{Na} = \bar{N}_a/\bar{N}_0$; $R_{Nb} = \bar{N}_b/\bar{N}_0$. Dependences $N_a(t)$, $N_b(t)$ and $N_0(t)$ for the blood sample of group B(III) are presented in fig. 5. Therefore the resolution value RN varies in the ranges from ~ 0 for the negative agglutination reaction to 10000 for the positive agglutination reaction. Blood saturation for the positive agglutination reaction (fig. 5, curve *3*) is explained by the fact, that within time interval, in which the saturation is observed, all pixels of zone W had brightness above Bbound, i.e. 10000 pixels. Values of resolution are presented in fig. 3.

5.2.3. Comparison of summary brightness of photo image pixels for positive and negative agglutination reactions Technique of summary brightness calculation is an improvement of the technique of pixels number count (sect. 5.2.2.). Indeed, the resolution of the previous technique can be additionally increased, if not only pixels number, brightness of which exceeds the set boundary value B_{bound}, but these pixels brightness is also considered. It is obvious, that areas S_- and S_+, indicated in fig. 4, *b* both *4* and *5*, physically show the summary pixels brightness for the cases of positive and negative agglutination reactions respectively. Designation S — is a summary brightness. It is easily seen (fig. 4, *b*), that at the negative agglutination reaction not just the number of pixels, for which $B \geq B_{\text{bound}}$ is low, but their brightness is also low, and as a result the summary brightness of such pixels S_0 is low.

At the same time for the positive agglutination reaction the number of pixels with brightness $B \geq B_{\text{bound}}$ is high and, moreover, their brightness is significant. Therefore for that case the summary brightness of all pixels S_+ exceeds the value of S_0. Then the resolution of AOM of blood typing with similar photo images processing $R_S = S_+/S_0$ can be high.

Values of the resolution were defined as a relation of the time-averaged values of S_{N0}, S_{Na}, S_{Nb} the following way: $R_{SNa} = \overline{S_{Na}}/\overline{S_{N0}}$; $R_{SNb} = \overline{S_{Nb}}/\overline{S_{N0}}$. Dependences of S_{N0}, S_{Na}, S_{Nb} on observation time are presented in fig. 6.

By comparing the diagrams of fig. 5 and 6, it is easy to notice the clear similarity in the curves pattern, but the numerical values in fig. 6 are much higher, that indicates the advantage of the technique of the resolution calculation through the summary brightness of pixels. The limit value of resolution in these two techniques is defined with the pixels number, i.e. the size of the experimental results processing zone W.

Table 3. Example of blood typing of the blood samples

Group of blood	Techniques of photo images computer processing									
	№ 1 Photometric tecnhique (sect. 5.1)		№ 2 Dispersion analysis of distribution of pixels		№ 3 Boundary value of brightness (sect. 5.2.1)		№ 4 Summary brightness of pixels (sect. 5.2.2) (sect. 5.2.3)		№ 5 Density of distribution of pixels by brightness (sect. 5.2.4)	
	R_a	R_b	R_a	R_b	R_a	R_b	R_a	R_b	R_a	R_b
0(I)	0.41	0.43	0.2	0.2	1.0	1.0	0.4	0.4	0.2	0.2
0(I)	0.80	0.92	1.2	1.2	1.0	1.0	0.8	0.9	0.9	1.0
A(II)	1.77	1.14	412.6	0.9	10000.0	1.2	1980000.0	1.4	33.7	1.2
A(II)	2.74	1.05	320.5	1.1	10000.0	1.0	2539924.2	1.1	46.6	1.1
B(III)	0.84	1.67	3.0	790.8	1.0	10000.0	0.8	1980000.0	1.4	44.6
B(III)	0.87	1.55	1.5	355.0	1.0	8378.4	0.9	33836.8	1.1	28.2
AB(IV)	2.20	2.20	652.0	652.0	10000.0	10000.0	2540000.0	2539971.4	53.7	53.7
AB(IV)	2.54	2.53	390.8	390.8	10000.0	10000.0	2539886.7	2532801.8	47.0	46.9

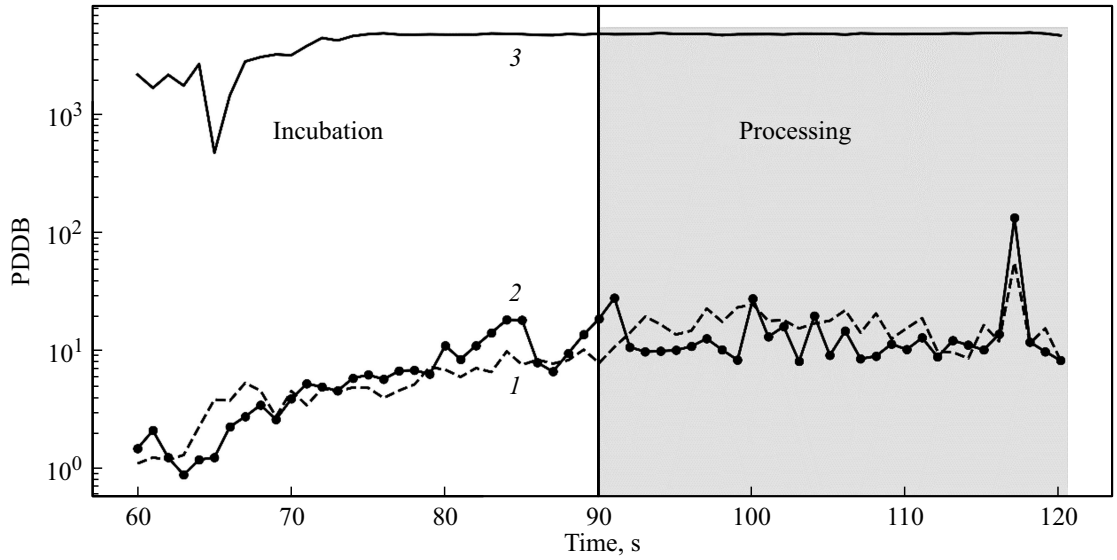

Figure 7. Dependences of pixels distribution density by brightness PDDB in the zone W on observation time: *1* — $PDDB_0$, *2* — $PDDB_a$, *3* — $PDDB_b$.

5.2.4. Determination of the average pixels distribution density by brightness This technique of photo images processing is also based on the analysis of pixels distribution for the selected zone W by brightness. As seen from fig. 2 and 4, *a*, for positive agglutination reaction almost all pixels have the same brightness value, while at the negative reaction the pixels distribution by brightness is close to normal. I.e. the pixels brightness spread for the positive reaction is significantly narrower, than for the negative reaction, and in the most cases it even equals to 1. Therefore the authors introduced the parameter, designated as the „pixels density distribution by brightness" PDDB (ρ_N) $\rho_N = N_{\max}/\Delta B$, where $N_{\max}$ — maximum in pixels distribution, and ΔB — distribution width at its half-height. Of course, ρ_N for the positive reaction $\rho_{+N} = N_{+\max}/\Delta B_+$ and the negative $\rho_{-N} = N_{-\max}/\Delta B_-$ have essentially different values.

Examples of dependences of PDDB values on observation time for one of the examined samples are presented in fig. 7. It is worth reminding that each moment of time corresponds to its own frame. Thus, for making a single kinetic curve in fig. 7, the dozens of photo frames had to be processed. Let?s also not forget, that three curves for three blood samples are presented here.

As seen in fig. 7, the „pure"sample and sample with the negative agglutination reaction correspond to the similar curves, average values of $\overline{PDDB_a}$ and $\overline{PDDB_0}$ are about dozens of units. At the same time for the sample with the positive reaction the value of $\overline{PDDB_b}$ reaches „saturation" rather fast and becomes equal to several thousands.

As in the previous sections, the resolution was determined as a relation of the averaged values the following way: $R_{\mathrm{PDDB}_a} = \overline{PDDB_a}/\overline{PDDB_0}$; $R_{\mathrm{PDDB}_b} = \overline{PDDB_b}/\overline{PDDB_0}$. Values of resolution of this technique are also presented in table 3.

Since the cases with „pure“ sample and the negative reaction are similar, the following conclusion can be made: if resolution value is low, in this case the agglutination reaction is negative (due to values similarity of $\overline{PDDB-}$ and $\overline{PDDB0}$). If resolution value is on the level of 1000 and above, the agglutination reaction is positive (value of $\overline{PDDB+}$ exceeds $\overline{PDDB0}$ by several orders).

6. Discussion of results

Number of blood samples, group identity of which had to be determined using AOM, was 34: 0(I) — 12 samples, A(II) — 7, B(III) — 8, AB(IV) — 7. It should be noted, that the authors had the complete information on the examined blood samples, including their group identity, from the start. The task was reduced to typing using AOM and the results comparing with the passport data. Computer processing of the photo frames using five different techniques (sect. 5) showed, that numerical values of „relative“ resolutions, by which the conclusion should be made on group identity of this blood sample, are essentially different at different techniques of the same photo frames processing. The results of such analysis and the corresponding criteria are presented in table 2.

Parameters of photo frames processing, as well as numbers of sections, where the processing technique name and its description are presented, are specified in column 1 of table 2. Actual maximum numerical values of the „relative“ resolution for the negative reaction on totality of 34 examined blood samples for all four static techniques of photo frames processing are presented in column 2. For identification of the blood group for the presented sample based on data from column 2 of table 2 the criteria R_{cr} (index cr means criterion), presented in column 3 of table 2, were set in computer program. These criteria were set with some margin, i.e. $R_{\mathrm{cr}} > (R_-)_{\max}$. Data from this column should be understood the following way: if „relative“ resolution R_a, calculated, for instance, as a parameter D, exceeds $R_{\mathrm{cr}} = 15$, then the reaction should be considered positive. Then the „relative“ resolution R_b corresponds to the negative reaction. It is more complicated for blood samples 0(I) and AB(IV). Therefore the algorithm of application of table 2 was the following.

1) If $R_a > R_{\mathrm{cr}}$, $R_b < R_{\mathrm{cr}}$, the sample belongs to blood group A(II). R_a is taken as the resolution value.

2) If $R_b > R_{\mathrm{cr}}$, $R_a < R_{\mathrm{cr}}$, the sample belongs to blood group B(III). R_b is taken as the resolution value.

3) If $R_a > R_{\mathrm{cr}}$, $R_b > R_{\mathrm{cr}}$, the sample belongs ti blood group AB(IV). Minimum of R_a and R_b is taken as the resolution value.

4) If $R_a < R_{\mathrm{cr}}$, $R_b < R_{\mathrm{cr}}$, the sample belongs to blood group 0(I). Minimum of R_a and R_b is taken as the resolution value.

Photo frames of videos for 34 blood samples were processed using five different techniques (sect. 5) with criteria table application (table 2). Due to the article volume limitations we present only part of the observed results for blood samples of different groups (table 3).

The following is seen from the table.

1) Photometric technique (№ 1) does not have the required resolution for the blood sample group determination. It is easily seen, for instance, from the fact, that for blood samples A(II) and B(III) the values of the „relative“ resolutions R_a and R_b should be significantly different, preferably by several orders, but in reality they were of the same order. Besides, parameters Ra and Rb for samples 0(I) and AB(IV) should be vastly different, since the sample 0(I) should not principally interact with tsoliclones of both types, while sample AB(IV), on the contrary, gives the agglutination reaction with both anti-*A* and anti-*B* tsoliclones. However, this is bot observed in table 3: values of Ra(0(I)) and Rb(0(I)) are close to values of R_a(AB(IV)) and R_b(AB(IV)).

2) At the same time four static techniques of photo images processing (№ 2–5) give positive result — the values of R_a and R_b are properly correspond to the presence or lack of agglutination reaction. For instance, for sample 0(I) the values of R_a and R_b ae close to 1 (no agglutination), while for the sample AB(IV) the same parameters increase to hundreds (technique № 2), thousands (№ 3) and even $> 10^6$ (№ 4) — agglutination reaction occurred. Of course, the bigger the difference between R_a and R_b, the higher reliability of the blood sample group identification. And only for the technique № 5 „the relative“ resolutions are in order of dozens.

3) It is fair to note, that the complete identification of blood groups for the examined samples using AOM was possible due to the fact, that for each technique of photo images processing the criteria for values of „relative“ resolutions were defined (table 2). Numerical values of criteria in table 2 are defined using 34 blood samples, this number increase can result in correction of table 2. Otherwise due to blood samples variability the errors in typing are possible.

4) At the same time such correction is natural. The thing is that during development and production of diagnostic devices, particularly, for blood typing, the manufacturers use reliability „monitoring“ in determination of blood type in clinics and blood centers. This is required for the further device improving. Thus, for instance, the results of testing of the device, developed by authors of [23], for blood type determination using AB0 and Rh systems, were compared to the results of parallel blood typing device Technicon Auto Analyzer, and in some cases for control with „manual“ (not instrumental) method. The comparison showed, that in 97.3% of 10042 blood samples both devices precisely determined the blood samples groups, and only in 266 cases

the devices response was „the group is not determined“. In these cases the blood group was elaborated manually, while using the reverse cross testing method. Another example: in the work [24] the device of Inverness Blood Grouping System (IBGSystem) was subject to test, similar to [23], where results, observed using this device, were compared to „manual“ method. Only in three cases of 2051 the result of blood group determination by IBGSystem devices differed from the results of manual blood typing.

5) And finally, it should be emphasized, that AOM of the blood sample group identity determination, examined here, is technically reduced to three photo images sampling (two with tsoliclones of anti-*A* and anti-*B* type, and the „pure“ sample). At the same time the method allows several techniques (preferably static) of these photographs processing. If all results lead to a single conclusion on the blood sample group identity, then with great probability it can be affirmed, that typing was successful. However, if during at least one technique the result was different, then the additional studies are required, for instance, using cross-method, but within AOM.

7. Conclusion

AOM of instrumental determination of blood samples group identity using AB0 system with application of tsoliclones of anti-*A* and anti-*B* types is experimentally tested. To improve a certainty in blood group determination the introduction of „reference“ sample — solution of the examined blood sample without tsoliclone („pure“ sample) as an additional test is proposed for the first time. Such sample a priori imitates the negative erythrocytes agglutination reaction, thus contributing to precise identification of actual negative erythrocytes agglutination reaction relating to the positive.

It is demonstrated, that blood typing certainty depends not only on the corresponding study technology, but also on the techniques of erythrocytes agglutination photo images processing. For the first time for AOM of blood typing the parameter of the „average pixels density distribution by photo image brightness“is proposed to use as a method resolution. Comparison of blood typing certainty for four options of static techniques of experimental results processing is presented. Criteria for „relative“ resolutions of agglutination reaction types, as well as their application algorithm are determined. Application of the developed criteria and the algorithm allowed to determine blood group for 100% of the examined samples. In general, this work can be considered as a stage of the further development of AOM of instrumental human blood typing.

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