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APPLICATION OF THE GDV-GRAPHY TECHNIQUE FOR THE ESTIMATION OF ANTIGEN-ANTIBODY REACTION

Stepanov A, Sviridov L, Korotkina SA, Achmeteli GG, Kriganivski EV.
Research Center, St. Petersburg, Russia

At the present moment the spheres of application of Gas Discharge Visualization (GDV) technique in biology and medicine are widely searched. Basing on the well-known fact that this technique can detect changes of physico-chemical characteristics of solutions of non-organic substances and biological liquids, we made an attempt to study the possibility of application of gas discharge glow for the registration of specific interaction of an antigen with a complimentary antibody – the so-called agglutination reaction. This very fact defined the aim of the present investigation.

In order to realize this aim the authors developed and applied the method of estimation of the characteristics of gas discharge glow around a drop of liquid. The method assumed that the drop of liquid should be pressed out of a disposable insulin syringe and placed in its end. Each sample was tested 10 times with the frequency of 30 shots a second and the duration of influence of the electromagnetic field – 10 seconds. Summary data of the GDV tests were compared with the results obtained by standard visual method.

Specific antibodies were received by the way of immunization of rabbits by ovalbumen or tularemic vaccine and the white mice – by specific vaccine. Apart from that the blood serum of a person recovered from the infection caused by conditionally-pathogenic bacteroid B.fragilis was used.

In order to carry out the main experiments it was first of all necessary to optimize the scheme of GDV analysis. Particularly, the following question had to be solved: was it necessary to stir the antigen-antibody complex laid-down on the bottom of the test tube? This part of the work was performed using the following complementary pairs of reagents:

- Ovalbumen + antibodies (blood serum of rabbits immunized by albumen);
- Vaccine strain of the specific virus+ antibodies (immunoglobulin against virus, obtained from ascetic liquid of white mice, inoculated with the corresponding vaccine);
- Tularemic antibody + antibodies (blood serum of rabbits inoculated with tularemic vaccine).

Using the abovementioned systems similar data were received. It turned out that even when the sediment (agglutinate) was carefully stirred the results of GDV analysis didn't agree with those of the visual test; moreover, they often contradicted the latter.

In case of GDV investigation of the supernatant when the sediment wasn't previously stirred, the results coincided with the data of visual registration of agglutination reaction. We suppose that under positive reaction the antigen-antibody complex formed sediment and the supernatant "got cleaned" from the components of reaction. When agglutination was not performed, the components of reaction were found in the suspension state, i.e. no cleaning (clarification) of the supernatant took place. The GDV technique registered visible (clarification of supernatant) and invisible (change of physico-chemical properties) differences in experimental and control (known negative) samples appearing at that; thus the technique indicated positive agglutination reaction.

Considering these data detailed experiments proving this generally formulated aspect were carried out further. One of these experiments was performed with the application of the

following reacting components:

1.Human blood serum with antibodies to B.fragilis in titer 1:640 (serum N 1);
2.Human blood serum without B.fragilis antibodies (serum N 2);
3.Suspension B.fragilis (antigen);
4.Physiological solution (0,85 % solution NaCl).

The scheme of experiment is shown in table 1.

Table 1

Scheme of experiment

<table>
<tr><td rowspan="3">1*
0,85 % NaCl</td><td rowspan="3">2
0,85 % NaCl + B.fragilis</td><td rowspan="3">3
0,85 % NaCl + antibodies to B.fragilis</td><td rowspan="2">Interspecific (complimentary) components of reaction</td><td>Combination of components in 24 h before investigation</td><td>4
0,85 % NaCl+B.fragilis +blood serum N1</td><td>5
0,85% NaCl+B.fragilis+ blood serum N1 in dilution 1:1000</td></tr>
<tr><td>Combination of components right before investigation</td><td>6
same</td><td>7
same</td></tr>
<tr><td>Internonspecific (non-complimentary) components of reaction</td><td>Combination of components in 24 h before investigation</td><td>8
0,85 % NaCl+B.fragilis +blood serum N2 in dilution 1:10</td><td>9
0,85 % NaCl+B.fragilis+ blood plasma N2 in dilution 1:1000</td></tr>
</table>

Comment: * - shows the number of sample.

Investigation of samples 1,2 and 3 (physiological solution NaCl, the same solution with B.fragilis or blood serum N1, respectively) was aimed at determining if the GDV technique could disclose such microscopic objects as antibodies and microorganisms in the solution.

Comparative assessment of GDV-grams of these three objects determined that sample 1 reliably differed from sample 2 in the intensity of glow and in background area, and from sample 3 – not only in these criteria, but also in the form coefficient and length of isoline. Samples 2 and 3 reliably differed from one another in all the abovementioned parameters. To our mind, all that indicates that the applied method enables to disclose if such biological objects as microorganisms, being the model of antigen in this case, and immune serum, containing specific antibodies to these microorganisms, are present in physiological solution. Moreover, differences between the blood serum and suspension of microorganisms can be revealed.

The results of assessment of samples 4-9 should have answered the main question: could the GDV technique identify not only antigen or antibodies to it in the physiological solution, but also specific reaction between them – agglutination reaction? This is possible theoretically, as new biological structures (immune complexes) are formed at such interaction. These structures possess different physico-chemical properties as compared to other initial reagents (antigens and antibodies), and most probably change the characteristics of the solution.

The main (pilot) sample of all was sample 4, which consisted of human blood serum with antibodies to B.fragilis, diluted by physiological solution up to titer 1:10, and suspension B.fragilis. Agglutination reaction was inevitable in such a system in view of specificity of the biological reagents taken, which was visually proved in 24 hours (when GDV-grams were taken).

Control sample 5 differed from sample 4 only by the fact that it had blood serum diluted at 1:1000. In this case no agglutination reaction took place, as the serum dilution (1:1000) exceeded its maximal titer (1:640).

Comparing GDV-grams of samples 4 and 5 it was found that they significantly differ in the intensity of glow. The only difference between them consisted in the fact that agglutination

reaction took place in one sample (sample 4), and was absent in the other (sample 5), which fact proved that the GDV technique could register new physico-chemical state of sample 4, which took place as a result of specific interaction of antigen and antibodies in it. In other words, the technique could visualize the agglutination reaction.

The following aspects prove this conclusion, as well:

- reliable differences in the intensity of glow (fig.1) between samples 4 and 6 (there was no agglutination in sample 6 as a result of insufficient exposure within the mixture of components: the components had been mixed right before the GDV-grams were taken, i.e. agglutination reaction hadn't yet taken place);
- reliable differences in the background area (fig.2), intensity of glow (fig. 3) and length of isolines (fig.4) between the samples 4 and 8 (agglutination in sample 8 didn't take place because of the fact that there was no specific antibodies to B.fragilis in blood serum N2);
- absence of differences between samples 6 and 7 (agglutination was absent in both samples because of insufficient exposure of antigen with antibodies);
- absence of differences between samples 8 and 9 (agglutination didn't take place in both samples as a result of heterologicality of antigen and antibodies).

Thus, as follows from the data above, comparative analysis of GDV-grams of pilot and control samples enables to reveal antibodies in the investigated material.

It was also considered important to study the informativeness of the GDV as a method of titration of immune sera. Special experiment on the determination of titer of antibodies in the blood serum of rabbits, inoculated with ovalbumen, was planned for that. The scheme of the experiment is represented in table 2.

Table 2. Results of titration of the blood serum of rabbits, inoculated with ovalbumen

NN of samples	1	2	3	4
Dilution of serum	1:10	1:20	1:40	1:4000
Titer of antibodies according to the data of visual assessment	++++	+++	+	-

Comparative analysis demonstrated that the GDV-grams of samples 1 and 2, similar in visually registered agglutination (4+ and 3+ respectively), didn't differ reliably. At the same time, sample 1 reliably differed in area and intensiveness of glow from sample 3 (agglutination +), and in area, intensity of glow, and also in the quantity of fragments (fig. 5) from sample 4, where agglutination was absent at all. Analogous differences were found comparing GDV-grams of sample 2 with the characteristics of sample 3 and 4. At the same time, computer analysis of samples 3 and 4, where agglutination was expressed weakly (sample 3) or was absent at all (sample 4), didn't reveal differences between them.

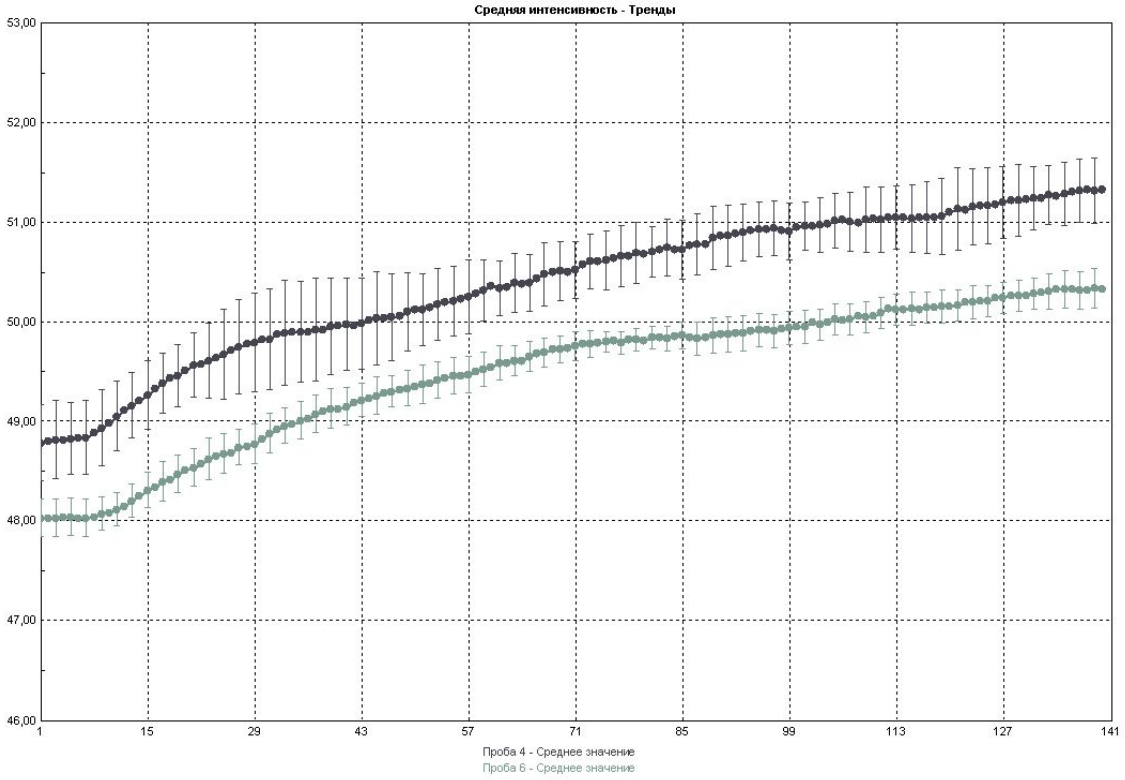
Therefore, the titer of antibodies determined by visual and GDV methods turned out to be the same: 1:20. This fact forms the basis that the GDV technique gives an opportunity not only to register antibodies in liquids, but also to determine their titer.

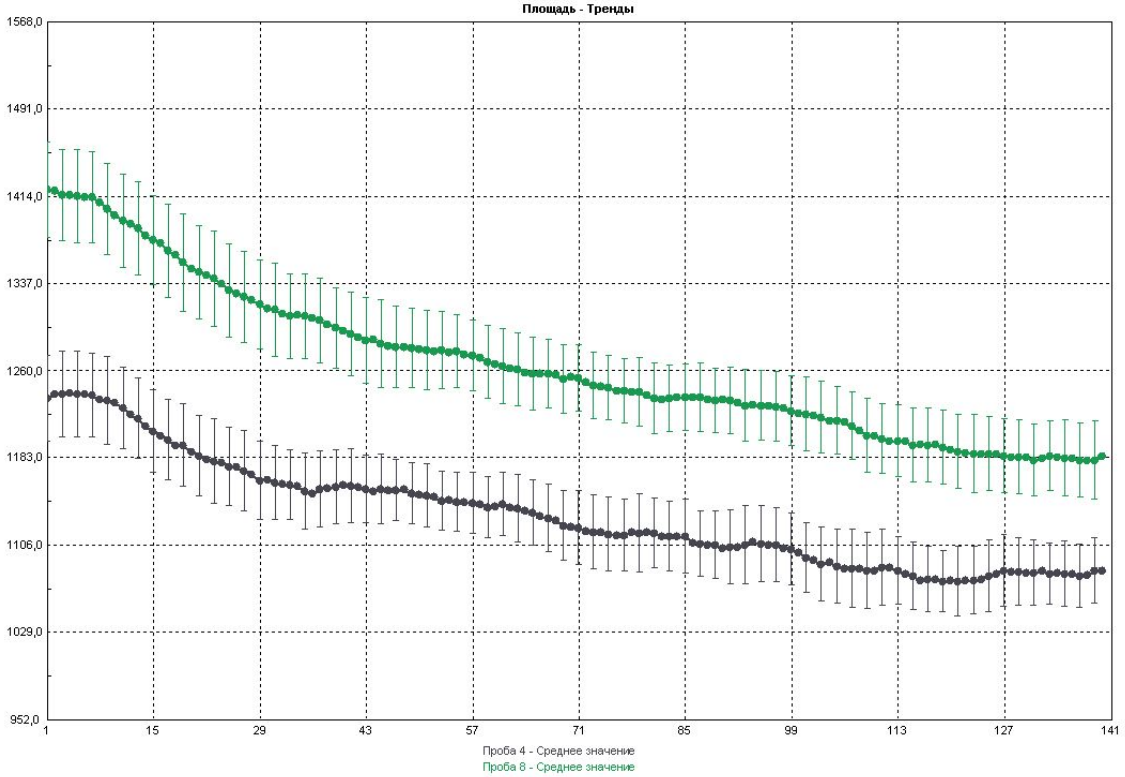
CONCLUSION

Basing on the presented materials we can conclude that the GDV technique enables to identify specific reaction of antibodies with a complimentary antigen, called agglutination reaction. The technique is based on the registration of dynamics of parameters of gas discharge glow in time – from the moment of combination (mixing) of specific components (antigen and antibodies to it) to the moment of completion of their interaction and formation of the so-called immune complexes. As a results of such interaction, physico-chemical characteristics of the investigated material, and consequently, GDV-gram parameters change.

The results obtained are of course preliminary and need to be specified further. But already now we realize the significance of this direction of scientific search.

The technique can be applied for the investigation of nontransparent biological liquids when it is not only difficult, but even impossible to implement the agglutination reaction in its classical form (visual registration of results); for example, researching the blood with the purpose of revealing etiology of human allergies.

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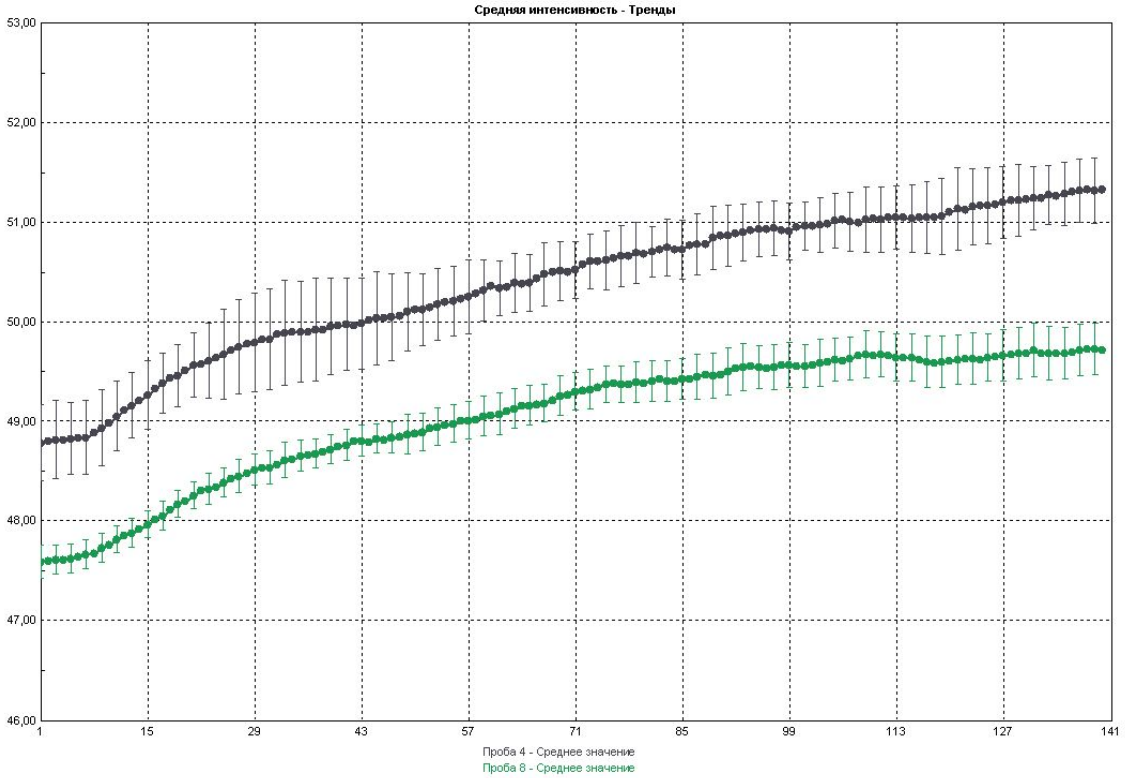
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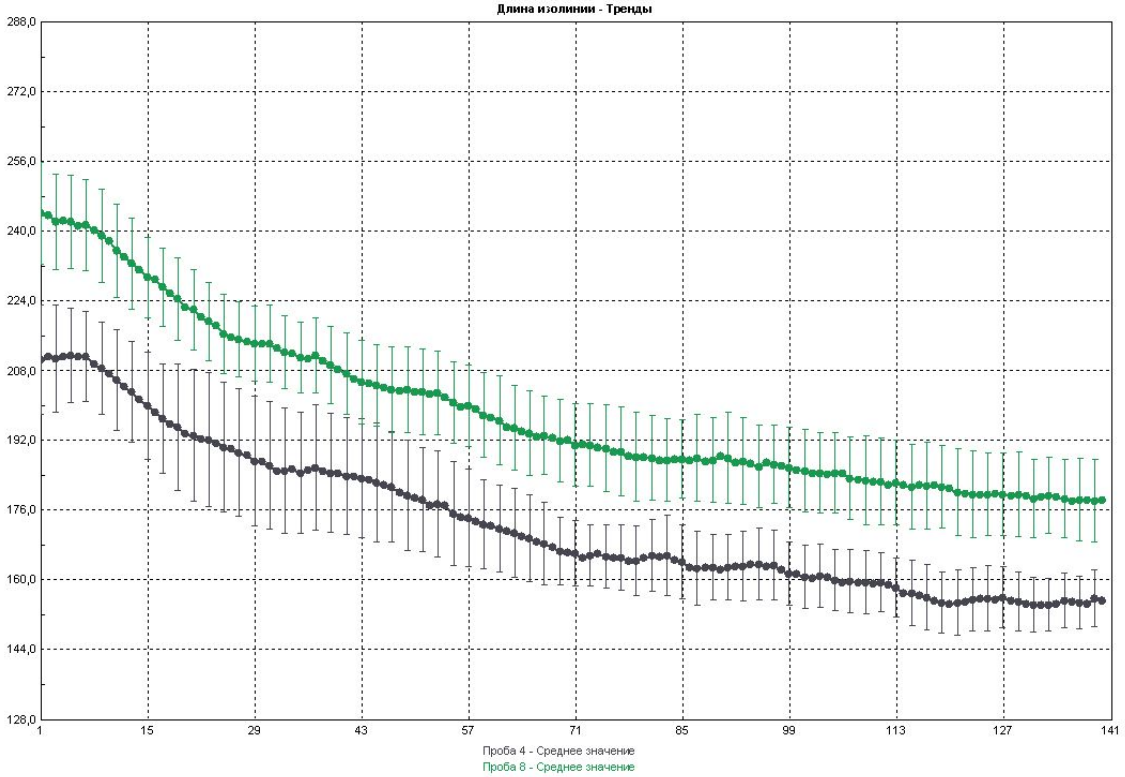
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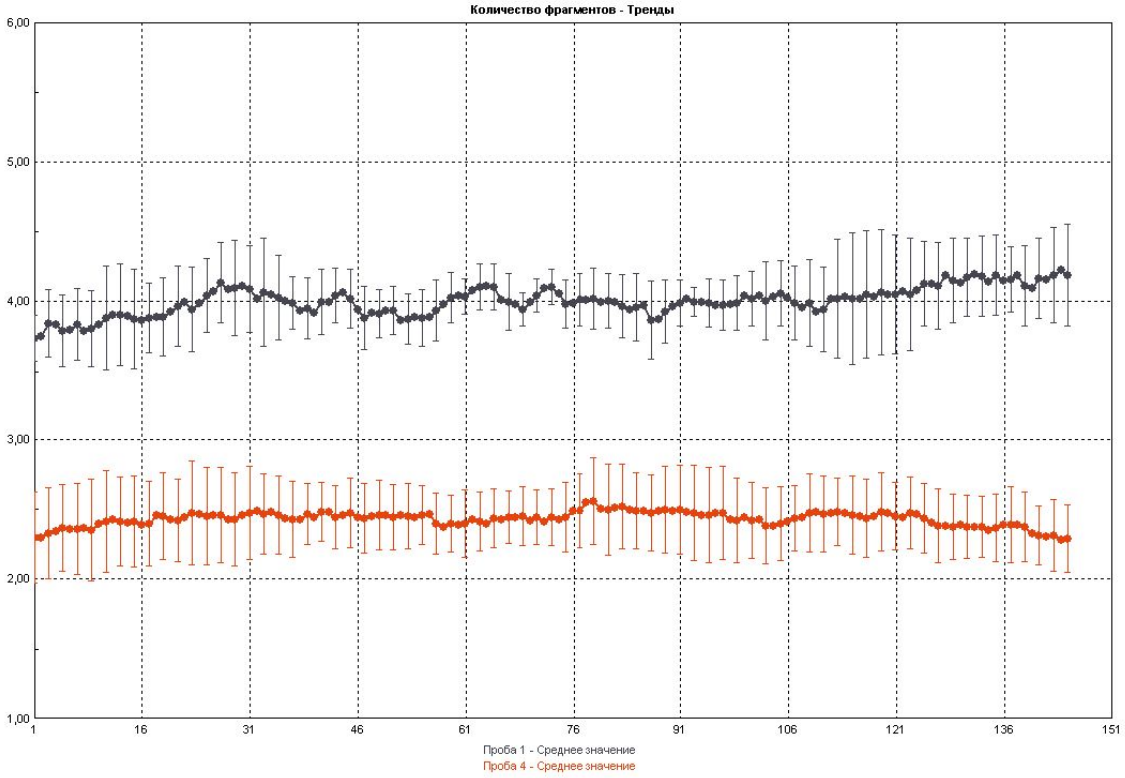
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