Application of SPR Analysis for Detection of Specific Antibodies in Human Blood Serum

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Introduction

According to the World Health Organization, viruses of the Herpesviridae family infect 90% of the Earth's population. Herpes simplex virus type I (HSV-1) is the most prevalent from those, which establishes latent infection but reactivates during low immunity, causing cutaneous or genital herpes, conjunctivitis and diseases both central and peripheral nervous system such as encephalomyelitis, polyneuropathies and others. Epstein-Barr virus is no less dangerous to human health. This virus is causing infectious mononucleosis, lymphoproliferative diseases and it may be involved in the formation of tumors.

Current diagnostic methods of HSV-1 and EBV infections include ELISA and PCR. Significant advantages of biosensor analysis are that it does not require any label, is performed in a short period of time and has high sensitivity.

The aim of our work was to develop biosensor chip for detection of specific antibodies to HSV-1 and EBV in the human blood serum.

Materials and Methods

Herpes simplex virus type 1 (strain UC) was accumulated in culture of epithelial cells MDBK provided by the Institute of Organic Chemistry with Center for Phytochemistry of Bulgarian Academy of Sciences. Virus was purified by differential centrifugation in density gradient of cesium chloride was followed by standard procedure of the disintegration to release the virus capsid proteins. Specific activity of viral antigen was estimated by indirect ELISA using commercial serum to HSV-1 ("Dako", Denmark).

ELISA of human blood sera was performed by using the test system "HSV-1 IgG ELISA" (GenWay, USA) according to the manufacturer's instruction.

Blood sera of patients with herpes infection and polyneuropathy were provided by Kyev hospitals. Blood sera of healthy donors were given by Blood transfusion station (Kyev, Ukraine).

Epstein-Barr virus was accumulated and purified from a B95-8 cell culture of marmoset, which produce this virus, and was prepared by the method Walls and Crawford [1] with our modifications [2].

Blood serum samples of patients with lymphoproliferative diseases and infectious mononucleosis with EBV-etiology were kindly provided by clinical center "DNA Lab" (Kiev, Ukraine).

Blood sera samples of healthy donors were provided by Blood transfusion station (Kyev, Ukraine).

Polymerase chain reaction. PCR test system "AmpliSens 100-R» (Russian) was used to determine the presence of EBV DNA in the test sera . The primers were 290 bp nucleotide sequences from VCA protein gene. The analysis was performed according to the manufacturer's instruction.

SPR analysis was carried out using the two-channel optoelectronic spectrometer "Plasmon-6“.

Results and Discussion

Surface plasmon resonance measurements were carried out with the optoelectronic two-channel spectrometer "Plasmon-6“ (Fig. 1), using the SPR phenomenon in the Krechman optical configuration. It was developed at the Lashkarev Institute of Semiconductor Physics of NAS of Ukraine and was provided for research under the program "Research in the field of sensor systems and technologies" of the National Academy of Sciences of Ukraine. Source excitation is GaAs laser, λ = 670 nm. Used carrier was a glass plate covered with 45 nm gold film.

Fig. 1. Optoelectronic spectrometer "Plasmon-6 “

Preparation of biosensor chips

Biochips for detection of antibodies to HSV-1 and EBV were prepared by the methods developed in our laboratory.

Chips were purified with “piranha” mix (water – H2O2 – HCl in proportion 5:1:1) for 15 min and three times were rinsed by distillated water and citrate buffer (pH 5.0-5.5).

To immobilize viral proteins 0.2% solution Dextran 17 000 (Sigma,USA) (for HSV-1) or 1% solution Guanidine Thiocyanate (Sigma, USA) (to detect antibodies to EBV) was applied to the surface of the chip and was kept for 18 hours at room temperature. Chips were rinsed three times by citrate buffer and antigen (viral proteins), diluted to the appropriate concentration in citrate buffer, was applied. To immobilize antigen chips were kept at 4-8˚C for 24 hours. Chips were rinsed three times by citrate buffer and were treated with 1% bovine serum albumin in citric buffer for 1 hour at room temperature for blocking unoccupied sites. Then BSA solution was removed.

Chips were thoroughly dried in the air, placed in a container and stored at 4-8˚ C.

Biosensor analysis

The obtained biochips were used for SPR analysis of specific antibodies to HSV-1 in the blood serum of people.

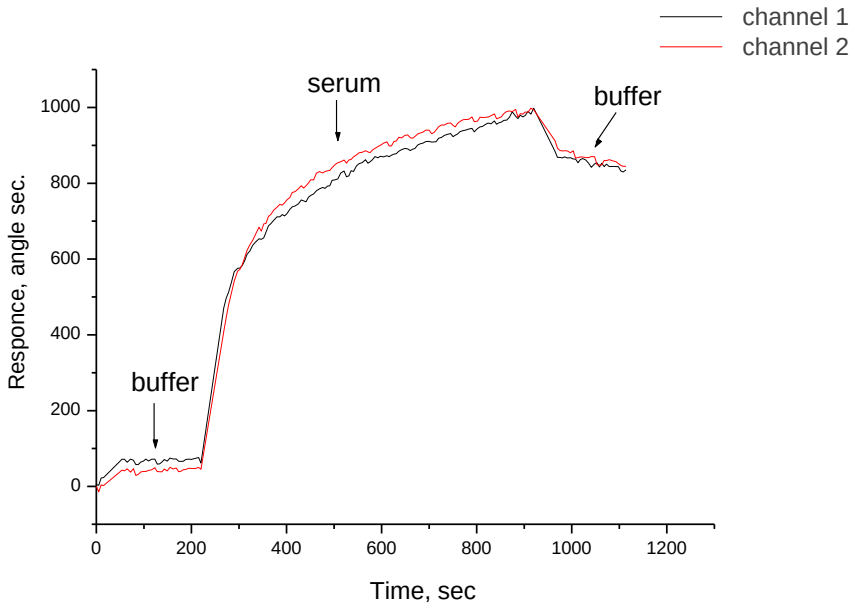
The limits of positive and negative feedback for SPR-analysis of specific antibodies to HSV-1 was determined by using pannel of the donor's blood sera which did not contain antibodies to HSV-1(on ELISA results). These were 185 a.s. ± 65 a.s. for this series of chips (mean plus two standard deviations). Thus, serum. which in SPR-analysis had a value greater than 250 a.s., was considered positive, and the serum. which gave feedback lower than 250 a.s., was respectively negative.

40 blood sera of patients were tested by ELISA and SPR. Serum samples had different of load of specific to HSV-1 antibody and on results of ELISA were divided into 4 groups: negative, weakly positive, positive and highly positive. ELISA values for negative sera did not exceed 0.385 OU, weakly positive sera had values within 0.385 - 0.465 OU, positive - in the range of 0.465 to 1.0 OU and highly positive sera, respectively, had values above 1.0 OU.

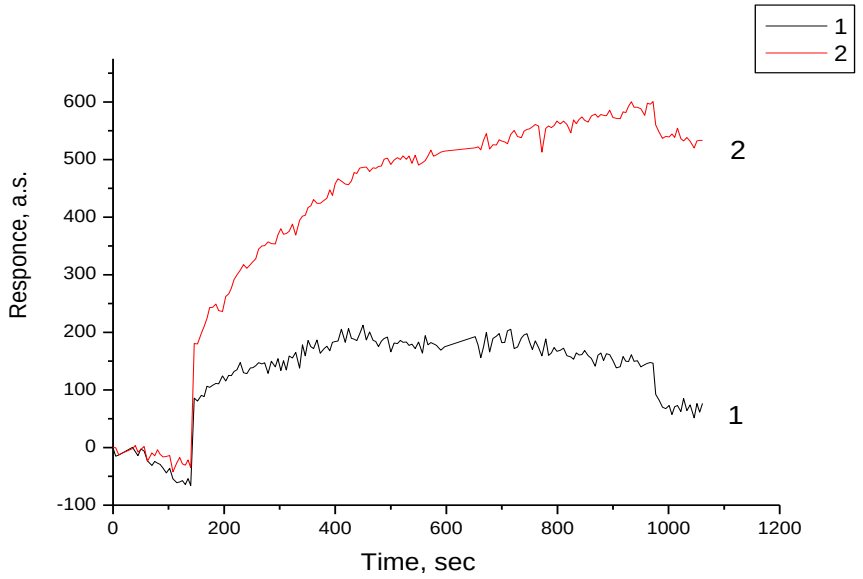
All sera were tested at least three repetitions by SPR analysis.

Sensograms of positive and negative to HSV-1 sera are demonstrated on Fig. 2.

Results of comparative analysis of blood sera by ELISA and SPR are presented in Tables 1-4.



A



B

Fig. 2. Typical sensograms of SPR analysis
A -positive to HSV-1 blood serum in two channels ;
B - negative (channel 1) and positive (channel 2) blood sera.

Sera were diluted 1:100 in citrate buffer (pH 5,0). Diluted serum samples were injected into both flow cells at a flow rate of 10 µl/min during 10 min.

Table 1. Determination of HSV-1 specific antibodies by SPR in group of weakly positive sera

№	Serum no.	SPR results (HSV-1 serostatus by SPR)	ELISA results
1	103	400 ± 15 к.с. (positive)	0,3785 ± 0,034 OU
2	148	447 ± 32 к.с. (positive)	0,4580 ± 0,010 OU
3	169	406 ± 41 к.с. (positive)	0,4430 ± 0,055 OU
4	187	368 ± 36 к.с. (positive)	0,3945 ± 0,012 OU
5	203	407 ± 30 к.с. (positive)	0,4565 ± 0,012 OU
6	239	433 ± 42 к.с. (positive)	0,4555 ± 0,024 OU
7	247	423 ± 36 к.с. (positive)	0,4143 ±0,003 OU
8	801	457 ± 56 к.с. (positive)	0,3595 ± 0,055 OU

Table 2. Determination of HSV-1 specific antibodies by SPR in group of positive sera

№	Serum no.	SPR results (HSV-1 serostatus by SPR)	ELISA results
1	101	723 ± 37 a.s. (positive)	0,4865 ± 0,043 OU
2	102	883 ± 86 a.s. (positive)	0,5180 ± 0,033 OU
3	125	556 ± 51 a.s. (positive)	0,4700 ± 0,041 OU
4	136	512 ± 26 a.s. (positive)	0,7061 ± 0,066 OU
5	175	526 ± 58 a.s. (positive)	0,6600 ± 0,041 OU
6	182	619 ± 62 a.s. (positive)	0,6325 ± 0,056 OU
7	199	907 ± 89 a.s. (positive)	0,7831 ± 0,072 OU
8	213	812 ± 83 a.s. (positive)	0,6070 ± 0,071 OU
9	221	787 ± 67 a.s. (positive)	0,5285 ± 0,015 OU
10	237	515 ± 55 a.s. (positive)	0,5235 ± 0,049 OU
11	254	680 ± 62 a.s. (positive)	0,9270 ± 0,094 OU

Table 3. Determination of HSV-1 specific antibodies by SPR in group of highly positive sera

№	Serum no.	SPR results (HSV-1 serostatus by SPR)	ELISA results
1	96	804 ± 89 a.s. (highly positive)	1,5735 ± 0,071 OU
2	119	863 ± 49 a.s. (highly positive)	1,050 ± 0,062 OU
3	139	487 ± 51 a.s. (positive)	1,3690 ± 0,148 OU
4	185	837 ± 59 a.s. (highly positive)	1,286 ± 0,082 OU
5	156	846 ± 78 a.s. (highly positive)	1,0835 ± 0,042 OU
6	229	1062 ± 84 a.s. (highly positive)	1,098 ± 0,073 OU
7	240	1264 ± 72 a.s. (highly positive)	1,414 ± 0,048 OU
8	241	989 ± 92 a.s. (highly positive)	1,148 ± 0,012 OU
9	259	840 ± 72 a.s. (highly positive)	1,244 ± 0,118 OU
10	262	788 ± 81 a.s. (highly positive)	1,177 ± 0,021 OU

Table 4. Determination of HSV-1 specific antibodies by SPR in group of negative to HSV-1 sera

№	Serum no.	SPR results (HSV-1 serostatus by SPR)	ELISA results (HSV-1 serostatus by ELISA)
1	121	**220 ± 21 a.s. (negative)**	**0,2070 ± 0,015 OU (negative)**
2	149	**195 ± 27 a.s. (negative)**	**0,2060 ± 0,012 OU (negative)**
3	173	**154 ± 31 a.s. (negative)**	**0,1850 ± 0,017 OU (negative)**
4	184	**197 ± 18 a.s. (negative)**	**0,2520 ± 0,018 OU (negative)**
5	186	**167 ± 18 a.s. (negative)**	**0,2565 ± 0,014 OU (negative)**
6	191	**166 ± 22 a.s. (negative)**	**0,2435 ± 0,011 OU (negative)**
7	198	**223 ± 21 a.s. (negative)**	**0,3660 ± 0,011 OU (negative)**
8	226	**170 ± 18 a.s. (negative)**	**0,2455 ± 0,018 OU (negative)**
9	147	**773 ± 74 a.s. (positive)**	**0,2715 ± 0,026 OU (negative)**
10	152	**667 ± 29 a.s. (positive)**	**0,2662 ± 0,017 OU (negative)**
11	787	**472 ± 51 a.s. (positive)**	**0,1805 ± 0,011 OU (negative)**

All sera, that according to ELISA belonged to the group of weakly positive sera, were also positive on results of SPR analysis. A feedback for majority of weakly positive sera ranged from 368 a.s. to 457 a.s. which is above the limit value of less than 2 times (Table 1).

11 sera, that according ELISA were attributed to a group of positive, were also positive in biosensor analysis. A feedback values were in limits from 512 a.s. to 907a.s. (Table 2).

In the group of highly positive sera (on results ELISA) responses of 7 sera of patients in SPR analysis ranged from 788 a.s. and above, except one serum (№139), which is although had a positive result, but it was too low for this group sera (Table 3).

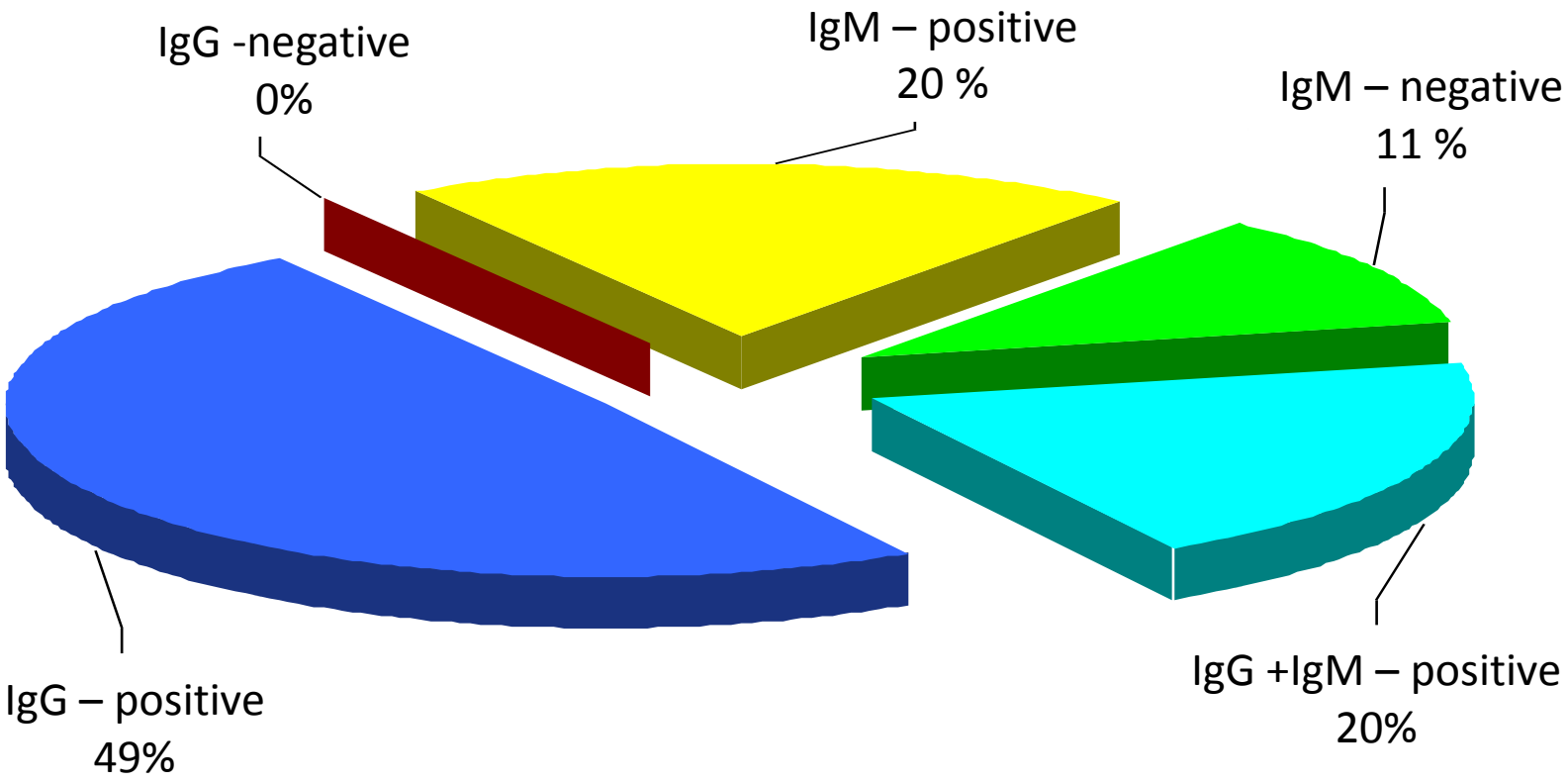
11 blood sera of patients on ELISA results were determined to negative. In this group 8 serum samples were also negative according to SPR analysis, but 3 serum showed positive results in SPR (Table 4). The cause of the discrepancy may be presence of early antibodies to HSV-1, namely IgM, which usually occurs in the primary stages of virus infection. Additional study of these sera for detection of early serum antibodies will allow to analyze the results in more details.

In our previous work [1] it was presented the results of the creation of specific biosensor chips for the detection of antibodies to EBV with using of viral antigen. Sera of patients with increased content of EBV antibodies were tested. It was shown the specificity and reproducibility of results of SPR analyses at using developed biochips which were the basis for this work.

Blood sera samples were tested by ELISA for detection antibodies to EBV using anti species antibodies against human immunoglobulins G and M. This procedure allowed differentiation of disease because the sera were obtained from patients at different stages of disease. The results are shown in Fig. 3. In the sera of all 17 examined patients were revealed immunoglobulins of class G to EBV, in 11 from them - antibodies of both classes.

Titer of IgG antibodies ranged from 1:40 to 1: 500, titer of IgM antibodies - from 1:10 to 1:640, which obviously dependent from the phase of viral infection. 10 selected donors sera which were negative to EVB by PCR showed absorbance values below the cut off by ELISA. Thus, sera from patients and donors were estimated by ELISA on the presence of antibodies to EBV and titers of specific antibodies were determined.

Fig. 3. Results of the study of blood sera of patients for the detection of antibodies to EBV by ELISA



By using SPR analysis all serum samples were detected in three replicates at dilutions 1:32 and 1:64. This range of dilutions of sera was chosen based on the fact that each subsequent serum dilution step reduces the signal doubled. Level of recorded values in response to the introduction of the test sera were in the range of 85 to 2980 arcsecunds. Graphic image of registration response for antibodies to EBV of one of the sera in flow mode is shown in Figure 4.

Results of the testing of all sera samples are shown in the Table 5. It was found that in healthy individuals results coincided in 100%. Among the 17 tested sera of patients antibodies to EBV had not been registered in two sera samples which indicates the sensitivity of the method at the level of 92%. These data suggest the possibility of using biosensor chips developed for diagnosis of EBV infection.

Fig. 4. Sensogram of SPR analyses for detection of antibodies to EBV in the flow mode

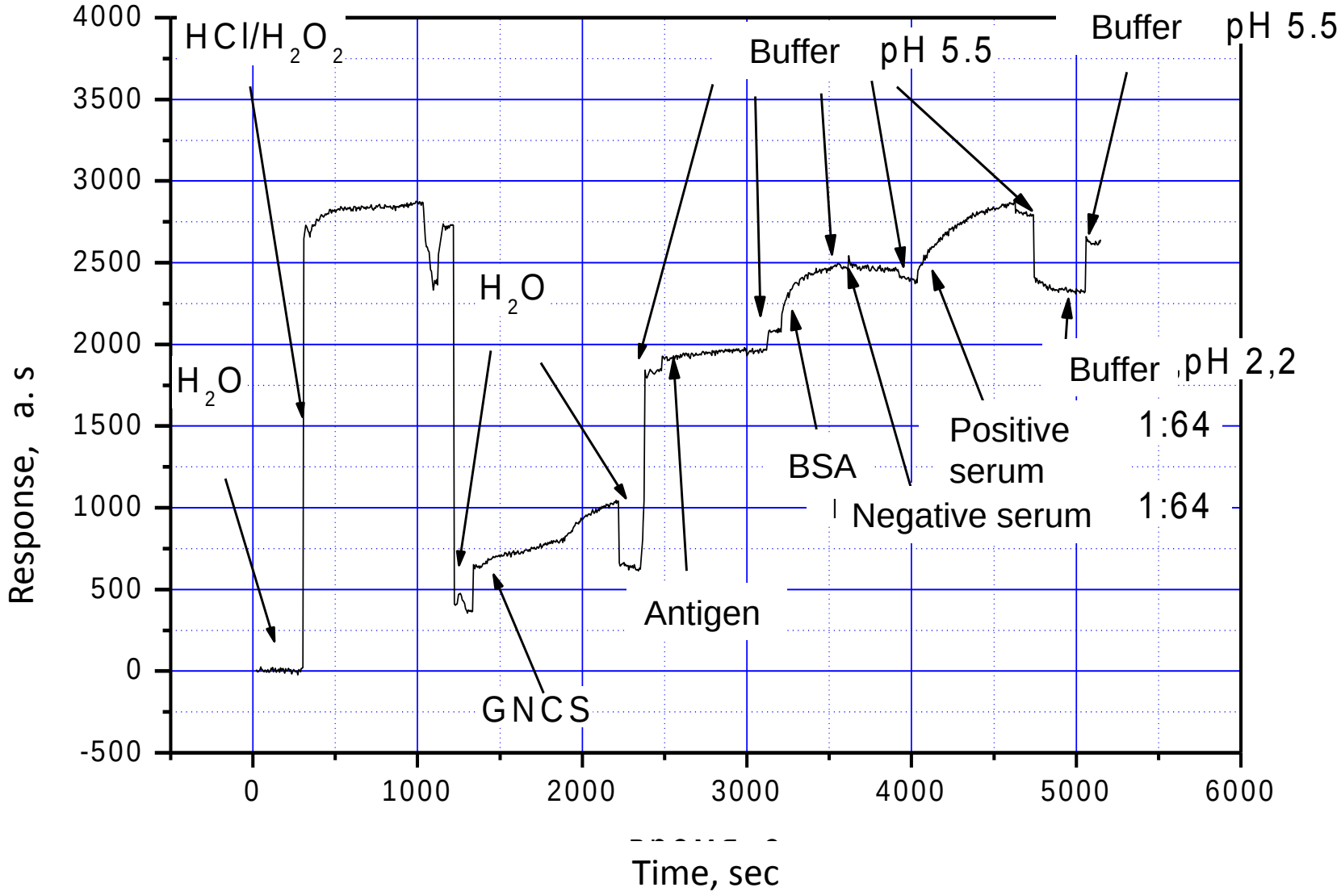


Table 5. **Results of detection of antibodies to EBV by SPR in the sera of patients and healthy donors**

	SPR results (arc seconds)*									
Serum dilution	**Healthy individuals (n=10)**									
1:32	90	110	96	110	110	115	85	175	90	150
1:64	35	85	82	97	85	97	60	118	70	90
Serum dilution	**Patients (n=17)**									
1:32	85	-	1070	400	500	1520	1280	2980	1340	
	630	110	2130	700	1320	680	2130	490		
1:64	65	-	518	125	270	600	990	1800	650	
	280	100	750	570	935	635	750	382		

*** Cut-off for the SPR assay was ≤ 370 a.s.**

Among the works on the use of SPR method for the detection of various biological macromolecules, the percentage of studies of clinical material, particularly the identification of specific antibodies in serum of patients, is very little [4-9]. It was carried out using SPR the diagnosis of bovine leukemia in the serum and the results were compared with the results of immunodiffusion reaction [4]. Based on these data the authors concluded that immunosensor analysis based on SPR is more sensitive, convenient and rapid method for the detection of antibodies than immunochemical techniques.

In study [5] it was shown the possibility of identifying hepatitis B surface antigen in the serum of patients using an optical biosensor. Comparative analysis of results of detection for HbsAg by optical biosensor and ELISA methods demonstrated high specificity of antigen detecting using a biosensor.

Analyzing the results of our research for detection of antibodies to EBV in the serum of patients and literature data it can be concluded about the possibility of using SPR method fpr analyses together with immunochemical methods, including ELISA. In addition, the SPR method has several advantages since the single-stage, does not require the use of labeled macromolecules can monitor and receive information on the course of the analysis. Thus, this method is quite promising for use in laboratory diagnosis of viral infections.

CONCLUSIONS

1.Laboratory model of biosensor chips for the detection of antibodies to HSV-1 and Epstein-Barr virus in human serum with using viral antigen were developed, the conditions of analysis were optimized.

2. The research of serum of patients with infection caused by Epstein-Barr virus (positive sera) and healthy individuals (negative) performed by SPR analyses and reference methods showed that the percentage of coincidence among negative sera was 100% and among 17 positive sera - 92%.

3. A comparative analysis of 40 blood sera of patients on detection the antibodies to HSV-1 carried out by ELISA and SPR with using developed biochips demonstrated the coincidence of the results for 37 of 40 cases (93%).

4. Immunosensor analysis using the device "Plasmon-6" allows to detect specific antibodies to HSV-1 and EBV in the blood sera as well as traditional approaches.

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