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Increasing the Efficiency of Laboratory Diagnostics of Infectious Diseases Using Innovative Technology of Ultrasonic Disintegration of Immune Complexes

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Abstract

The problem of increasing the efficiency of laboratory diagnostics of infectious diseases using innovative technology of ultrasonic disintegration of immune complexes is considered. The innovation of the technology lies in the application of ultrasonic action on the sample using a dispersant at the stage of preanalytical preparation in order to destroy circulating immune complexes, which leads to an increase in the amount of free antibodies and antigens in the sample. During the study, the technological parameters required for ultrasonic disintegration were determined. The results of evaluating the effectiveness of the application of the developed technology are presented. The results obtained confirm the effectiveness of the developed technology and make it possible to proceed to the creation of a new methodological approach for laboratory diagnostics. The potential area of application of the developed technology is diagnostic laboratories and blood storage and transfusion centers.

Key words*: ultrasound, circulating immune complexes, laboratory diagnostics, infectious diseases, antibodies, antigens.*

1. Introduction

Today, one of the most important tasks for modern society is the problem of timely diagnosis of infectious, oncological, autoimmune, allergic diseases in order to provide effective therapy at an early stage of the disease. One of the main examples of infectious diseases is HIV infection, which can progress to AIDS in later stages. According to the data [2] for 2019, 1.3 million HIV-infected people were registered in the territory of the Russian Federation. For 10 years, according to Rospotrebnadzor, the number of HIV-infected in Russia has tripled. According to the World Health Organization (WHO), Russia has come out on top in Europe in terms of the growth rate of HIV incidence. Russia has the highest infection rate - 71 cases per 100 thousand population. The most effective methods for containing the epidemic are effective prevention, early diagnosis of the disease and ensuring access to treatment for all people with a positive HIV status [3-5, 7-11, 21].

Early diagnosis of HIV infection makes it possible to prescribe therapy in a timely manner and to increase its effectiveness [1, 3, 16, 19]. The disadvantage of modern test systems for HIV infection is the

ability to diagnose the disease for more than several weeks, in the worst case, 1-2 months, during which a person is already a carrier of infection [11]. In accordance with the Michaelis-Menten law for the diagnosis of HIV infection, the ratio of the number of antibodies and antigens should be nonequivalent [5, 10-12, 16]. At the initial stage of the disease, circulating immune complexes are formed in the body, which are bound antibodies and antigens [14, 18, 23]. Thus, at an early stage of HIV, due to the lack of unbound antibodies and antigens (seronegative zone), diagnostics using existing methods (for example, enzyme immunoassay) leads to unreliable results [6, 13, 15-22, 24].

For early diagnosis of HIV infection, the destruction of circulating immune complexes will increase the number of unbound antibodies and antigens that can be registered using existing test systems, the sensitivity of which allows the determination of picograms of the substance. Destruction of the circulating immune complexes is possible if the complex transfers energy that exceeds the binding energy of the complex. An innovative approach for the destruction of the antibody-antigen complex is the transfer of ultrasonic energy to the sample.

The aim of the work is to increase the efficiency of laboratory diagnostics by increasing the amount of unbound antibodies and antigens in the sample due to the use of the technology of ultrasonic disintegration of immune complexes before the enzyme immunoassay.

During the study, the following tasks were set:

- To determine the optimal parameters for ultrasonic disintegration: the geometry of the waveguides and test tubes, the power and frequency of ultrasonic exposure, the duration of exposure to the sample;
- Tto establish the minimum sample volume for ultrasonic disintegration;
- To carry out experimental studies of improving the efficiency of diagnostics through the use of ultrasonic disintegration of immune complexes.

2. Materials and Methods

For the study, samples were obtained from 28 patients with a confirmed diagnosis of HIV, who were under dynamic dispensary observation at the AIDS Center of the Leningrad Oblast State Healthcare Institution "AIDS Center", St. Petersburg. Confirmation of the diagnosis was carried out by the State Budgetary Healthcare Institution "Center for the Prevention and Control of AIDS and Infectious Diseases", St. Petersburg, using an enzyme-linked immunosorbent assay, after which studies were carried out by the method of immune blotting. Patients were also examined with the quantitative determination of HIV-1 RNA by real-time PCR in order to administer and monitor antiretroviral therapy.

To carry out ultrasonic disintegration of immune complexes, an ultrasonic laboratory unit I100-840 (Russia) was used, which includes a generator of ultrasonic radiation with a frequency of 22 kHz and 44 kHz.

When developing the technology for disintegration of immune complexes, it was necessary to analyze the geometry of the waveguides in order to determine the most optimal one for efficient delivery of radiation to the sample. In the course of the work, the analysis of waveguides D1-D6 was

carried out, the characteristics of which are presented in Table 1.

Table 1 - Characteristics of Waveguides used for Ultrasonic Exposure

WaveGuideDimensions (mm)	WaveGuidename					
	D1	D2	D3	D4	D5	D6
Length	58	68	78	106	125	52
Endpartdiameter	25	15	6	25	15	38

The method for determining the optimal technological parameters consisted in varying the waveguides, the radiation frequency 22, 44 kHz, the generator power level from 10 to 100% in 10% steps, and the exposure duration from 30 to 300 s. After each experiment, the number of circulating immune complexes was recorded before and after ultrasound exposure.

To determine the optimal geometry of the test tube, studies of the containers presented in Table 2 were carried out.

Table 2 - Main characteristics of Tubes up to 2.5 ml

Name	Manufacturer	Material	Volume (ml)	Diameter (mm)	Height (mm)	Bottom	Sterility
Eppendorf	Aptaka S.P.A. (Italy)	Polypropylene	1,5	10,5	40	round	no
Eppendorf	Aptaka S.P.A. (Italy)	Polypropylene	2,0	11	44	round	no
Cryovialwithlid	Greetmed (China)	Polypropylene	1,8	11	45	round	no
Eppendorf	Aptaka S.P.A. (Italy)	Polypropylene	2,0	10	48	round	yes
Vacutainer	Guangzhou (China)	Polypropylene	2,4	13	100	round	yes
Skirtedcryovial	JetBiofil (China)	Polypropylene	1,8	11	41	round	yes

The method for choosing the optimal capacitance consisted in registering the possibility of contacting the waveguide to the sample.

Experimental studies of improving the diagnostic efficiency were carried out by assessing the amount of unbound antibodies (extinction level) before and after ultrasonic disintegration using the following test systems:

- ARCHITECT HIV Ag / AbCombo (Abbott, USA);
- NEWLAVBLOTI (Bio-Rad, USA);
- IFA-BEST (Vector-Best, RF);
- Abbott RealTime HIV-1 (Abbott, USA).

3. Results and Discussion

The results of determining the minimum sample volume are presented in Table 3.

Table 3 - The results of determining the minimum volume of the biological environment for ultrasonic disintegration of immune complexes using a dispersant

Tubename	Manufacture	Samplevolume (ml)	Minimumsamplevolume (ml)	Name of waveguides with parameters corresponding to the conditions of ultrasonic exposure
Eppendorf	Aptaka S.P.A. (Italy)	1,5	0,8	D3
Eppendorf	Aptaka S.P.A. (Italy)	2,0	1,0	D3
Cryovialwithlid	Greetmed (China)	1,8	0,9	D3
Eppendorf	Aptaka S.P.A. (Italy)	2,0	1,0	D3
Vacutainer	Guangzhou (China)	2,4	1,0	D3
Skirtedcryovial	JetBiofil (China)	1,8	0,9	D3

In the course of experimental studies of containers, it was found that when using containers with a limited volume of up to 2.5 ml, the optimal test tube is an Eppendorf test tube with a volume of 1.5 ml with a minimum sample volume of 0.8 ml. In this case, the D3 waveguide possesses the optimal geometry.

The results of determining the optimal frequency and power of radiation are presented in tables 4, 5.

Table 4 - CIC Content after Exposure at a Frequency of 22 KHz

Generatorp Ower (%)	The Content of the CIC after Ultrasound Exposure at a Frequency of 22 kHz using Dispersants D1-D6					
	D1	D2	D3	D4	D5	D6
10	90	89	88	85	91	89
20	90	88	85	86	89	87
30	88	86	81	85	86	85
40	85	79	76	82	74	83
50	80	69	68	76	67	79
60	76	69	68	77	68	79
70	76	69	70	74	67	78
80	72	67	69	74	68	78
90	70	68	68	74	68	78
100	70	67	68	73	68	77

Table 5 - CIC Content after Exposure at a Frequency of 44 kHz

Generatorp Ower (%)	CIC Content after Ultrasound Exposure at a Frequency of 44 kHz using Dispersants D1-D6					
	D1	D2	D3	D4	D5	D6
10	91	90	89	92	90	87
20	91	88	85	89	89	86
30	89	88	84	89	82	84
40	89	89	77	88	80	82
50	88	85	75	86	76	76
60	88	85	74	85	72	77
70	87	85	75	84	72	77
80	88	84	74	85	70	76

90	87	85	74	84	71	77
100	88	84	74	83	70	77

In the course of experimental studies, it was found that the radiation frequency of 22 kHz in the power range of 50-100% is the most effective. It should be noted that ultrasonic action on the sample leads to its heating, and therefore, the recommended value of the input power is 50%.

The results of evaluating the effectiveness of the technology are presented in tables 6, 7.

Table 6 - Characteristics of Extinction Levels, Positive for the Presence of Specific Antibodies to HIV in Blood Serum, before and after Ultrasound Exposure

No	Extinction level before ultrasound exposure (OD / cutoff)	Ultrasonicexposuretime (sec)	Installationpower (%)	Ultrasonicfrequencyrange (KHz)	Extinction level after ultrasound exposure (OD / cutoff)
1	2	3	4	5	6
1	4,765/0,254	180-300	100	22-44	4,944/0,254
2	4,749/0,254	180-300	50	22-44	5,957/0,254
3	4,862/0,254	180-300	50	22-44	4,971/0,254
4	4,642/0,237	180-300	50	22-44	4,652/0,237
5	5,044/0,237	180-300	50	22-44	5,083/0,237
6	4,685/0,237	180-300	50	22-44	5,940/0,237
7	0,5596/0,218	180-300	50	22-44	0,5781/0,218
8	0,4285/0,218	180-300	50	22-44	0,4437/0,218
9	4,895/0,226	180-300	50	22-44	>6/0,226
10	4,876/0,226	180-300	50	22-44	>6/0,226
11	4,528/0,226	180-300	50	22-44	>6/0,226
12	4,833/0,226	180-300	50	22-44	>6/0,226

Changes associated with an increase in the extinction of blood serum positive for the presence of specific antibodies to HIV were recorded in 12 out of 28 cases in the course of experimental studies. Table 6 shows that the greatest absolute increase in extinction is observed in samples 9-12, where the

result was obtained for the presence of antibodies, exceeding the upper threshold of the sensitivity of the test system (more than 6.0 extinction units). It is also worth noting a significant increase in serum extinction after ultrasound exposure in samples 2 (1.208) and 6 (1.255). In other cases, there was a slight increase in extinction after ultrasound exposure (from 0.010 to 0.179).

In 8 out of 12 samples, the upper threshold of sensitivity was not exceeded. These samples were analyzed for the absolute increase in extinction, as well as their relative (%) change, while the pre-exposure extinction levels and the cut off extinction level were used as a comparison (Table 7).

Table 7 - Characteristics of the absolute and relative magnitude of changes in extinctions, positive for the presence of specific antibodies to HIV in blood serum, before and after ultrasound exposure

No	Extinction level before ultrasound exposure (OD / cutoff)	Extinction level after ultrasound exposure (OD / cutoff)	Extinction gain after ultrasound exposure (abs)	Extinction gain after ultrasound exposure (total / cutoff,%)
1	4,765/0,254	4,944/0,254	0,179	3,75 / 70,47
2	4,749/0,254	5,957/0,254	1,208	25,43 / 475,59
3	4,862/0,254	4,971/0,254	0,109	2,24 / 42,91
4	4,642/0,237	4,652/0,237	0,010	0,25 / 4,22
5	5,044/0,237	5,083/0,237	0,039	0,76 / 16,45
6	4,685/0,237	5,940/0,237	1,255	26,79 / 529,53
7	0,5596/0,218	0,5781/0,218	0,185	3,31 / 84,86
8	0,4285/0,218	0,4437/0,218	0,152	3,54 / 69,72

In the course of the studies, the positivity threshold was crossed, showing an increase in extinction levels in relation to the cut off. The maximum in percentage terms are 475.59 and 529.53. These values are 4-5 times higher than the level required to confirm the diagnosis of HIV. The most significant is the result obtained with respect to sera, for which the upper threshold of sensitivity of the test system (more than 6.0 extinction units) was overcome, which confirms the increased efficiency of diagnosing HIV infection using ultrasonic disintegration.

4. Conclusion

In the course of the study, the problem of increasing the efficiency of laboratory diagnostics of infectious diseases with the help of ultrasonic disintegration of immune complexes was considered on the example of HIV infection. As a result of the study, the technological parameters necessary for the application of the developed technology were determined. Studies have been carried out, the results of

which confirm an increase in the level of extinction due to ultrasonic disintegration to diagnostically significant values, which, in turn, increases the efficiency of laboratory diagnostics.

The technology of ultrasonic disintegration of immune complexes is an innovative approach in laboratory diagnostics, the widespread use of which will increase the efficiency of diagnostics of infectious diseases at early stages

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References

Bartlett J. Clinical aspects of HIV infection 2012/ Bartlett J., Gallant J., Pham P. - M.: R. Valent, 2012 - 528 p.

Belyakov N.A. (2021) Virus immunodeficita ¬– opasnostdlycheloveka [The immunodeficiency virus is a danger to humans]. St. Petersburg Humanitarian University of Trade Unions. Saint Petersburg, 2020. Ser. 206 Selected lectures of the University.

Biomerieux. VIRONOSTIKA HIV Uni-Form II Ag/Ab: https://www.biomerieux.com/en/hiv-aids#The roleof in vitro diagnostics; 1998 [cited July 20, 2021].

Bio-Rad. Geenius HIV 1/2 Supplemental Assay: http://www.bio-rad.com/en-us/product/geenius-hiv-1-2-supplemental-assay?ID=NGUT8KE8Z; 2014 [cited 2021 July 20].

CDC/APHL. Laboratory testing for the diagnosis of HIV infection: updated recommendations: https://doi.org/10.15620/cdc.23447; 2014 [cited August 1, 2021].

Challakere K, Rapaport MH. False-positive human immunodeficiency virus type I ELISA results in low-risk subjects. West J Med. 1993; 159(2):214–5. PMID: 8212699; PubMed Central PMCID:PMC1022242.

Cohen, M.S., et al., Prevention of HIV-1 infection with early antiretroviral therapy. N Engl J Med, 2011. 365(6): p. 493-505.

Coleman SM, Gnatienko N, Lloyd-Travaglini CA, Winter MR, Bridden C, Blokhina E, etal. False-positiveHIV diagnoses: lessons from Ugandan and Russian research cohorts. HIV Clin Trials. 2018; 19(1):15–22. https://doi.org/10.1080/15284336.2018.1429846 PMID: 29384717; PubMed Central PMCID:PMC5949866.

Fauci, A.S., et al., Ending the HIV Epidemic: A Plan for America. JAMA, 2019. 321.

Fearon M. The laboratory diagnosis of HIV infections. Can J Infect Dis Med Microbiol. 2005; 16(1):26–30. Epub 2007/12/27. https://doi.org/10.1155/2005/515063 PMID: 18159524; PubMed Central PMCID:PMC2095005.

FinogeevYu.P. (2001) Clinical and laboratory diagnostics of infectious diseases [Kliniko-laboratornayadiagnostikainfektsionnykhbolezney]. Guidelines for doctors// FinogeevYu.P., Yu.V. Lobzin, Yu.A., S.M. Zakharenko [et al.]. - St. Petersburg, 2001. – 384 p.

Hill E. The importance of external quality assessment in HIV enzyme immunoassay testing: https://www.mlo-online.com/disease/hiv/article/13009399/the-importance-of-external-

quality-assessment-in-hiv-enzyme-immunoassay-testing; 2017 [cited July 20, 2021].

Klarkowski D, O'Brien DP, Shanks L, Singh KP. Causes of false-positive HIV rapid diagnostic test results. Expert Rev Anti Infect Ther. 2014; 12(1):49–62. https://doi.org/10.1586/14787210.2014.866516 PMID: 24404993.

Korolevskaya L.B., Shmagel K.V., Shmagel N.G. (2015) Kharakteristikatsir kuliruyushchikhimmunny kh kompleksov u VICh-infitsirovannykhpatsiyentov s razlichnymurovnemvirusnoynagruzki [Characteristics of circulating immune complexes in HIV-infected patients with different levels of viral load]. Bulletin of Experimental Biology and Medicine. 2015. Vol. 159. No. 4. pp. 460-463.

Lang R, Charlton C, Beckthold B, Kadivar K, Lavoie S, Caswell D, et al. HIV misdiagnosis: A root cause analysis leading to improvements in HIV diagnosis and patient care. J Clin Virol. 2017; 96:84–8. https://doi.org/10.1016/j.jcv.2017.10.005 PMID: 29031156.PLOS ONE False HIV positive results: Evidence of the importance of a confirmatory testing during HIV survey PLOS ONE | https://doi.org/10.1371/journal.pone.0239782 October 22, 2020 10 / 12

Lisitsyna Z.N., Dmitrievskaya K.A., Karaban N.V., Kondrashova T.V (2017) Immunnyye testy idiagnostikaostroystadii VICh-infektsii [Immune tests and diagnostics of the acute stage of HIV infection] // HIV infection and immunosuppression. 2017.- Vol.9, No. 2. - pp. 36-41.

Montesinos I, Eykmans J, Delforge ML. Evaluation of the Bio-Rad Geenius HIV-1/2 test as a confirmatoryassay. J Clin Virol. 2014; 60(4):399–401. https://doi.org/10.1016/j.jcv.2014.04.025 PMID: 24932737.

Ryazanova G.A., Koksin V.P., Khamzina R.V (2005) "Svobodnyye" i "svyazannyye" antitela k strukturnymbelkam VICh-1 v ranniy period zabolevaniya ["Free" and "bound" antibodies to HIV-1 structural proteins in the early period of the disease]. Medical immunology. 2005. Vol. 7. No. 1. pp. 73-76.

Saprygin D.B. (2016) Laboratornaya diagnost ikainfektsionny khbolezney [Laboratory diagnostics of infectious diseases]. Handbook // D.B. Saprygin, A.M. Ivanov - M., 2016– - 648 p.

Stamatatos, L., et al., Neutralizing antibodies generated during natural HIV-1 infection: good news for an HIV-1 vaccine? Nat Med, 2009. 15(8): p. 866-70.

UNAIDS/WHO. UNAIDS/WHO Working Group on Global HIV/AIDS/STI Surveillance. Guidelines forusing HIV testing technologies in surveillance: selection, evaluation and implementation– 2009 update:http://www.who.int/hiv/pub/surveillance/hiv_testing_technologies_surveillance_.pdf; 2009 [cited August 1, 2021].

USAID. Performance of Enzyme Immunoassays for HIV Serology in Surveys conducted by the Demographic And Health Surveys Program: https://dhsprogram.com/pubs/pdf/CR39/CR39.pdf; 2016 [cited2021 July 26].

sVzorov A.N. (2020) Immuno biologia HIV I osobennosti immunno gootvetaprotiv HIV-1 [HIV immune biology and features of the immune response against HIV-1]. textbook [for students of the Faculty of Biology of Moscow State University]. - Moscow : MAKS Press, 2020. - 87 p.

Wesolowski LG, Delaney KP, Lampe MA, Nesheim SR. False-positive human immunodeficiency virusenzyme immunoassay results in pregnant women. PLoS One. 2011; 6(1):e16538. https://doi.org/10.1371/journal.pone.0016538 PMID: 21304592; PubMed Central PMCID: PMC3029371.

Singh, Jaspreet, and A. K. Pandey. "Clinical Evaluation of Puskarmula (Inula Racemosa) Capsule in the Patients Of Metabolic Syndrome." *International Journal of Medicine and Pharmaceutical Sciences (IJMPS)* 4.2 (2014): 9-20.

Diwakar, R. P., et al. "Current Update on Canine Parvovirus in Veterinary Field Aspect." *International Journal of Zoology and Research (IJZR)* 4.5 (2014): 71-82.

Hashem, Somaya, et al. "A simple multilinear regression model for predicting fibrosis scores in chronic Egyptian hepatitis C virus patients." *International Journal of Bio-Technology and Research* 4.3 (2014): 37-46.

Al-Ansari, Waleed Ibraheem Ali, Ali Shawki, and Thura Hikmat. "The Effectiveness of Tigecycline in Multidrug Resistant Infections In Intensive Care Unit." *International Journal of Medicine and Pharmaceutical Science (IJMPS)* 7 (2017): 13-20.

Ketaren, S., et al. "Environmental health aspect in health emergency management (a case study: Sinabung Vulcanous Eruption)." *Int. J. Appl. Nat. Sci* 5 (2016): 47-56.

Priya, T. "Antimicrobial Activity of Evovulus Alisinoids (L) Extract with Different Organic Solvents in Pathogenic Bacteria and Fungal Species." (2017). International Journal of Applied and Natural Sciences (IJANS) 6.6, Oct-Nov 2017; 47- 54