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LABORATORY METHODS FOR THE DETECTION OF MARKERS OF HEMOTRANSMISSIVE INFECTIONS IN BLOOD DONORS

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ABSTRACT

New laboratory diagnostic capabilities, as well as vaccination of the population against blood-borne infections and inactivation of the virus in donor plasma can reduce the risk of infections in recipients after transfusion. The article examines modern methods of virus inactivation to ensure the infectious safety of donor blood.

Introduction: There are two trends in the world among various methods to ensure the safety of blood component transfusions: a) Laboratory testing methods for transfusion-

transmissible infections in donor blood; b) Quarantine, which involves combining other technologies with the above method for virus inactivation. In 1974, the genome of the hepatitis B virus (HBV) was isolated using radioimmunoassay methods [1]. Starting from 1989, immunoassay test systems (ELISA) have been actively implemented [1, 2]. The technology for inactivating plasma using the Reagent/Detergent method proposed by American specialist Professor Horowitz B at the New York Blood Center in 1991 has been licensed in Germany [13, 14]. From this point on, the choice of technologies and methods to achieve the infectious safety of blood and its components began to differ. In Germany, Switzerland, and the Netherlands, a comprehensive approach was implemented: laboratory tests, virus inactivation, and since 1995, the quarantine of hemotransfusion products [6].

In Scandinavian countries, especially Finland and the USA, laboratory testing methods are considered the most fundamental [6, 10, 18, 20]. Viral inactivation was not widely used like quarantine. In plasma quarantine, only those blood donations with positive infection markers from primary screening tests are preserved. These doses are kept until the monitoring and confirmed test results are known [15].

The safety of blood components is ensured through a number of measures: • a survey questionnaire to detect the presence of various diseases and infectious agents in donors; • detection of various disease symptoms in donors as a result of a therapist's examination; • conducting laboratory screening for hemotransmissive infection markers and reviewing the medical restrictions database; • quarantining plasma; • performing polymerase chain reaction to detect the presence of viruses; • methods for inactivating viruses in plasma.

These measures are regulated based on various normative documents. Several large laboratories are equipped with serological and PCR screening analyzers, and some have a laboratory information system. However, often, low-quality test systems are used when checking the donors' blood.

It is difficult to completely avoid errors at all stages of laboratory testing. In this case, laboratories equipped with high-tech instruments, utilizing sensitive test systems, and operating with high-quality systems have a significantly lower probability of error.

Blood safety is ensured at a high level only in places where modern technologies of blood services (including leukofiltration methods) are applied. The use of modern 4th generation ELISA test systems and test systems for immunochemiluminescent analysis in the laboratory practice of blood services shortens the 'diagnostic window' period for the detection of hemotransfusion

infection markers. In general, the application of highly sensitive methods of serological screening ensures a high level of blood safety, but in global practice, there are still cases of recipients being infected with components that have negative results from serological tests.

The effectiveness of PCR methods depends on the sensitivity of the test systems used, and the threshold for detecting the virus may vary by up to 10 times. The variability in the concentration of TORCH infection viruses in the blood of infected individuals and the different sensitivities of diagnostic kits have led test system manufacturers to develop international standards to monitor the sensitivity of PCR tests. The establishment of international standards enabled the PCR test to be conducted in a mini pool that includes several donations rather than each donation individually [3, 4, 10]. NAT tests in mini pools are cost-effective as they significantly reduce time and material resources [4].

The creation of multiplex test systems specifically designed for donor blood PCR screening reduces the cost and duration of research [7]. The development of automated, semi-automated, and modular platforms for real-time donor blood PCR screening significantly increases the effectiveness of PCR screening by using multiplex test systems on individual samples in mini pools and production pools [19].

The error rate does not exceed 0.1% in laboratories equipped with high-tech instruments and information systems [21].

Quarantine undoubtedly increases the viral safety of plasma. At the same time, it is unrealistic to expect that every donor can be re-examined six months after donation. The introduction of PCR screening of donor blood, the reduction of the quarantine period and the reduction of the time for repeated PCR testing of donors to 4 weeks will allow detecting the infection during the virological window and will reduce economic losses from quarantine storage of plasma [16,17].

The use of the quarantine method will ensure the viral safety of blood components to a certain extent, but the use of modern highly sensitive PCR methods can significantly increase the viral safety of blood transfusions.

In addition to HIV and hepatitis B and C viruses, many other viruses and microorganisms, such as herpes group viruses, parvovirus B19, human T-lymphotropic virus, syphilis, malaria, toxoplasmosis, and other pathogens, have the potential to cause infection during blood transfusion. The laboratory screening of these infection markers automatically increases the price of blood components [12].

Broad-spectrum systems that sterilize viral and bacterial pathogens in blood components disrupt the replication processes of bacteria and viruses. Currently, the method

of virus inactivation is widely used in blood centers of many countries such as Australia, Austria, Germany, the Netherlands, Greece, Italy, Spain, Canada, Norway, Portugal, Switzerland, and Japan. In European countries, virus-inactivated plasma and platelet concentrates are used in pediatrics, transplantology, as well as in obstetric and surgical operations that require large-volume plasma transfusions [12].

Thus, the method of virus inactivation allows for the deactivation of plasma that has not been quarantined from viral and bacterial pathogens.

Purpose of the study. Analysis of methods for testing hemotransmissible infections in donor blood.

Research methods: This study conducted a retrospective analysis of donors who voluntarily donated blood to the Republican Blood Transfusion Center of the Republic of Uzbekistan in 2021-2023.

Results and Discussion of the study: The quality of blood samples for infectious diseases in the territory of the Republic of Uzbekistan was studied for the years 2021-2023 (1 - figure). The results of the analysis of blood samples for HIV, hepatitis B, and C showed that the rate of refusals for positive results increased by 10.4% for hepatitis B and by 17.7% for hepatitis C, while the HIV infection rate decreased by 6.9% ($p<0.01$) (table 1). The increase in the proportion of blood samples with improper ALT and AST levels was 25.9% ($p<0.01$) (table 2). The proportion of improper samples for syphilis (RW) and brucellosis increased by 16.5% and 7.9% respectively ($p<0.01$) (table 3). These indicators are summarized in table 4.

Table 1 Disability due to hemotransmissive infection in the Republic of Uzbekistan

		2021	2022	2023	Total
Number of blood donors (plasma)		236732	257 734	296 180	790646
HIV	Number of examinations	237707	396 801	303 666	938174
	Positive results	361	281	336	978
	Determinability , %	0,15	0,11	0,11	0,12
	Blood disposed of according to the act	174,94	131,71	164,23	470,88

Hepatitis «B»	Number of examinations	237707	396 327	303 673	937707
	Positive results	6996	7489	7 725	22210
	Determinability , %	3,0	2,9	2,6	2,8
	Blood disposed of according to the act	3500,1	3669,9	3742,68	10912,68
Hepatitis «C»	Number of examinations	237707	396 327	303 666	937700
	Positive results	3121	3104	3673	9898
	Determinability , %	1,3	1,2	1,2	1,3
	Blood disposed of according to the act	1502,32	1503,91	1710,46	4716,69

Table 2 Unsuitability for ALT, AST indicators

		2021	2022	2023	Total
Number of blood donors (plasma)		236732	257 734	296 180	790646
ALT, AST	Number of examinations	237868	266 125	276 063	780056
	Positive results	3259	3252	4 104	10615
	Determinability , %	1,4	1,3	1,4	1,3
	Blood disposed of according to the act	1372,78	1384,16	2104,6	4861,54

Table 3 Unsuitability due to bloodborne infection

		2021	2022	2023	Total

Number of blood donors (plasma)		236732	257 734	296 180	790646
RW	Number of examinations	237707	396 327	303 666	937700
	Positive results	2063	2170	2403	6636
	Determinability , %	0,87	0,84	0,81	0,84
	Blood disposed of according to the act	968,91	1062,18	1156,13	3187,22
Brucellosis	Number of examinations	237868	266 125	304 023	808016
	Positive results	585	651	631	1867
	Determinability , %	0,25	0,25	0,21	0,24
	Blood disposed of according to the act	279,5	321,21	337,54	938,25

Table 4. Disability due to hemotransmissive infection in the Republic of Uzbekistan, %

Blood donation time		2021	2022	2023	Total
Number of blood donors (plasma)		236732	257 734	296 180	790646
Determinability , %	HIV	0,15	0,11	0,11	0,12
	Hepatitis «B»	3,0	2,9	2,6	2,8
	Hepatitis «C»	1,3	1,2	1,2	1,3
	ALT, AST	1,4	1,3	1,4	1,3
	RW	0,87	0,84	0,81	0,84
	Brucellosis	0,25	0,25	0,21	0,24

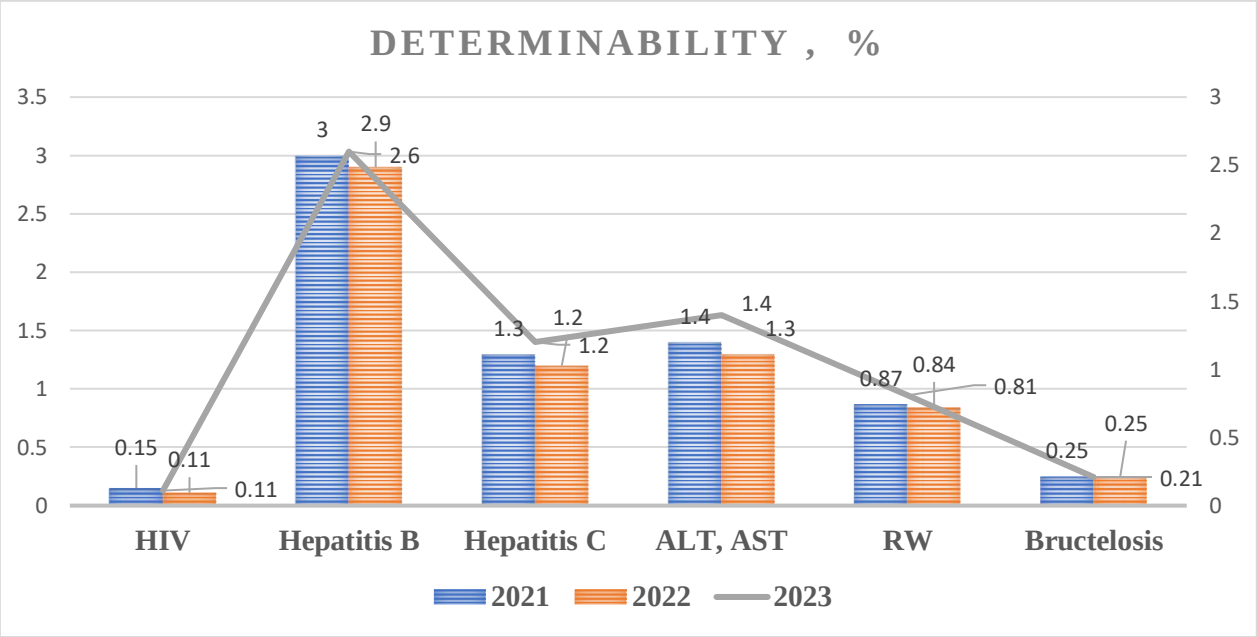

Fig. 1. Disability due to hemotransmissive infection in the Republic of Uzbekistan

From 2021 to 2023, the rejection rate at the Republican Blood Center increased by 9.8% ($p<0.01$) (Table 5). In particular, rejections based on specific markers of viral hepatitis increased as follows: HBV by 26.5% ($p<0.01$), HCV by 42.2% ($p<0.01$), and HIV infection increased by 62.3% ($p<0.01$). Rejections based on signs of syphilis increased by 10.3% ($p<0.01$). Brucellosis infection increased by 37.5% ($p<0.01$), while the rejection rate for the "ALT and AST" column decreased by 5% ($p<0.01$) (2 - figure).

Table 5 Reasons for the unsuitability of donor blood in the Republican Blood Transfusion Center

Year	Surveyed donors	HIV		Hepatitis B		Hepatitis C		RW		ALT, AST		Brucellosis		Unusable samples	
		abs	%	abs	%	abs	%	abs	%	abs	%	abs	%	abs	%
2021	35 151	61	0,17	865	2,46	519	1,48	311	0,88	1350	3,84	48	0,14	3154	8,97
2022	37 277	58	0,16	1009	2,71	576	1,55	373	1	1139	3,06	51	0,14	3206	8,6

2023	38 595	99	0,26	1094	2,83	738	1,91	343	0,89	1283	3,32	30	0,08	3587	9,29
χ2	6,18	618,26		228,53		380,36		6,28		3,18		336,18		6,08	
p	<0,01	<0,01		<0,01		<0,01		<0,01		<0,01		<0,01		<0,01	

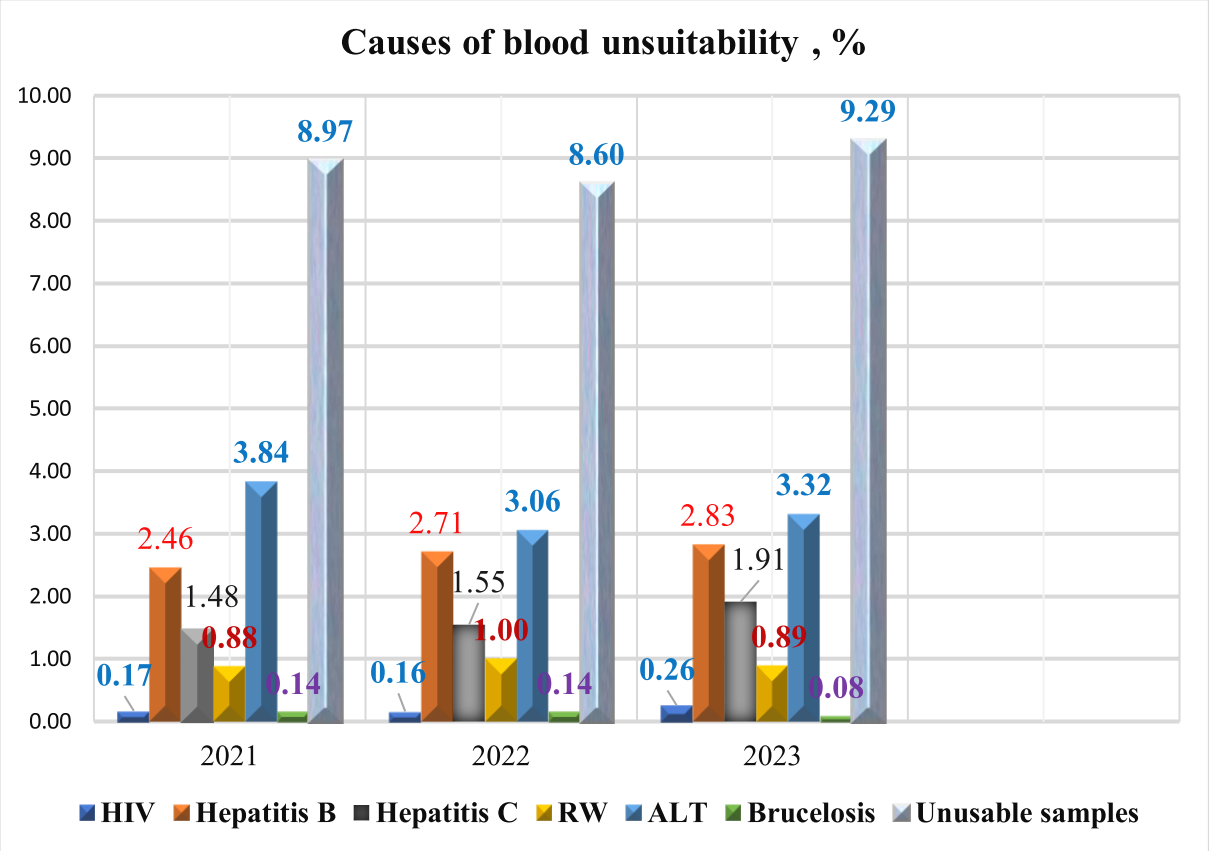

Fig. 2. Reasons for the unsuitability of donors blood

Conclusion: Thus, the laboratory methods used to check donor blood and the level of organization of laboratory services are often related to the viral safety of the prepared blood components. If there are diseases connected with herpes: HIV, tuberculosis, cancer, diseases of the hematopoietic system, donating blood for further use is prohibited.

At the Republican Blood Control Center, the number of tested donors in 2023 increased by 9.8 times compared to 2021. The introduction of two-stage laboratory tests for

ELISA and PCR contributed to an increase in the detection of HIV, hepatitis B, and C viruses by 1.6, 1.3, and 1.4 times, respectively. The total number of blood samples deemed unsuitable increased by 1.14 times.

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