«APPROVED»

 Director of NIIEMIZ MZ RUz

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 “21st” of June 2013

**TESTING RECORD**

*as to EIA testing of serum and lymphocyte content of donor blood*

*for HBV and HIV infection detection*

Under the agreement between “New Medical Technologies” limited liability company (General Director – Arifbaev R.S.) and Laboratory for chronic infection process research (Laboratory Head – Professor Gulyamov N.G., MD) of Scientific Research Institute for Epidemiology, Microbiology and Infection Diseases under the Ministry of Health of the Republic of Uzbekistan (hereinafter referred to as *“NIIEMIZ MZ RUz”*) (Institute Director – Professor Tuichiev L.D., MD), there has been issued the testing record No.2 of April 29, 2013 as to EIA tests of serum and lymphocyte content of donor blood for detecting of HBV and HIV infection among 309 donors subjected to testing.

 **Testing Objective:**

Functionality assessment of *“A method for detecting lymphotropic viruses, such as hepatitis B virus (HBV) and human immunodeficiency virus (HIV), in biological substrates having low content of viral particles, assessment of their viability, and exclusion of false-negative results of EIA testing”*. A comparative analysis of a rate of HBV and HIV marker detection in EIA testing of serum and lymphocyte content of donor blood.

**Preamble:**

The data of scientific reports by medical researches of CIS countries and Republic of Uzbekistan claim that from 30-40% to 60-80% of HBV and HCV infection cases related to human subjects, especially those related to children, including HIV infection cases, tend to occur as a result of transfusion of infected donor blood or infected blood-derived products, i.e. during medical manipulations. This is a direct evidence of deficiencies of existing testing methods currently used for examination of donor blood for detecting HBV and HCV infection and also HIV infection. However, no research work focused on discovery of reasons behind so frequent donor blood-related cases of HBV, HCV, HIV infections in human subjects had ever been done. More than that, such issue has never been under discussion yet (Gulyamov N.G., Babakhodzhaev S.N., Akhmedova H.Yu.// The diagnosing of viral infections in donors: EIA or PCR testing? Infections, Immunity and Pharmacology, No.3, 2005, p.p.113 – 115).

In addition, donor sites tend to reject about 4.5% of conserved donor blood based on the results of screening markers of hemotransmissive infections. In terms of distribution shares, donor blood rejection reasons are distributed as follows: HBV-related rejections are 20-25% and HCV-related rejections are 30-35%, and such distribution pattern also has a substantial economic significance (Onishenko G.G., Dement’eva L.A., The spread of viral hepatitis as a threat to national security, J.Microbiol., 2003, No.4, p.p.93-99).

 The key reasons behind a potential risk existing for donor blood recipients of their being infected with infections from blood components and blood-derived products are limited sensitivity and limited specificity of the testing methods used on donors to detect HBV and HCV infections (Gulyamov N.G., Babakhodzhaev S.N., Akhmedova H.Yu.// The diagnosing of viral infections in donors: EIA or PCR testing? Infections, Immunity and Pharmacology, No.3, 2005, p.p.113 – 115).

The occurrence rate of HBV, HCV, HIV infections in human population that provides potential blood donors remains high and may vary strongly all over certain regions. Viral hepatitis having parenteral transmission mechanism turns out to be the most frequent reason of post-transfusion infectious complications.

 There are some separate reports claiming that HBV and HCV, similarly to HIV, may be persistent in mononuclear blood cells, in particular in lymphocytes and macrophages. However, none of the researchers had even attempted to address this particular issue in terms of pathogenesis and clinical course of viral hepatitis.

The basis of doing this research was laid by simultaneously exhibited hepatotrophic and lymphotropic properties of viruses.

**Performed testing**

The present research work involves use of blood serum and lymphocytes of 309 blood donors. Blood sampling for research was performed from donors on the premisses of Mobile Blood Transfusion Station (MBTS) named “Uzbekistan Temir Youllari” (Chief Medical Officer - Babakhodzhaev S.N., Ph.D).

As a part of testing EIA testing of blood serum and content of lymphocytes was carried out using a testing system designed for specific diagnosing HBV and HIV by means of detection of HBsAg. During the testing, EIA of lymphocyte content was carried out on the basis of *“A method for detecting lymphotropic viruses, such as HBV and HIV, in biological substrates having low concentration of viral particles, assessment of their viability, and exclusion of false-negative EIA testing results”*. In order to execute EIA testing of lymphocyte content, donors were exposed to blood sampling in an amount of 5 – 6 ml taken under fasting condition from the basilic vein in the mourning using vials with 2.0 ml of sodium chloride isotonic solution and 2-3 drops of heparin. Lymphocytes were isolated by applying layers of heparinized whole blood onto ficoll-verografin solution having the density gradient d = 1,077 g/ml at a ratio 3:1, followed by centrifuging at 1500 rpm for 15 min. The lymphocytes thus isolated were washed out twice in normal saline for 15 min each time, using centrifuging at 1500 rpm. After the last centrifuging, a supernatant was removed. The precipitate comprising lymphocytes was diluted with 500 μl of normal saline and placed to Eppendorf vial. The isolated lymphocytes were destroyed by putting the Eppendorf vial to a freezer for freezing the lymphocytes at a temperature of -28OC for 24 hours. The method of detecting HBV and HIV antigens in lymphocyte content is identical to the method of detecting the same in blood serum.

In order to detect HBV and HIV by EIA testing in blood serum and lymphocyte content, testing systems manufactured by “VECTOR – BEST” company and “NPO Diagnosing Systems” company (Nizhniy Novgorod, Russian Federation).

The scope of testing was as follows:

1.EIA testing of donor blood serum to detect HBV infection…………………..309 tests

2.EIA testing of lymphocyte content to detect HBV infection………………….309 tests

3.EIA testing of donor blood serum to detect HIV infection……………………309 tests

4.EIA testing of lymphocyte content to detect HIV infection…………………..309 tests

In total, EIA testing performed………………………………………………….1236 tests

 The results of comparative analysis of how often HBV and HIV markers had been detected in donor blood serum and lymphocyte content allowed to establish the following. Out of 309 tested donors, 6 donors (1.94%) showed positive result of EIA testing of blood serum for HBV detection, whereas 23 donors (7.44%) showed positive result of EIA testing of lymphocyte content for the presence of HBV. At the same time, a difference between positive results of EIA testing of blood serum and lymphocyte content was 17 (5.5%) donors. This demonstrates that when it comes to EIA testing of donor blood serum a rate of false-negative results was 5.5% (Table 1).

 Analysis of a rate of identifying HBV-infected donors throughout various regions of the republic showed that the highest rate of HBV antigen identification was found in the donors from Andizhan – 20% of cases and in the donors from Cocand – 17.5% of cases. When it comes to the donors from Tashkent and Tashkent Region, these values were 3.50% and 3%, respectively.

 In case of EIA testing of blood serum for HIV infection detection, HIV antigen was detected in none of the donors tested (0%). Use of EIA testing of lymphocyte content for HIV detection showed positive (!) result of EIA testing in 1(0,32%) donor only.

Table 1

*Comparative analysis of HBV and HIV antigen detection rate*

*in serum and lymphocyte cytoplasm of EIA tested blood donors*

|  |  |  |  |
| --- | --- | --- | --- |
| No. | Location | Donors | Rate of HBV and HIV marker detection |
| HBV | HIV |
| Serum | Lymphocytes | Difference | Serum | Lymphocytes | Difference |
| 1 | Andizhan | 40 | 2(5%) | 8 (20%) | 6(15.0%) |  |  |  |
| 2 | Cocand | 40 | 2(5%) | 7(17.5%) | 5(12.5%) |  |  |  |
| 3 | Tashkent | 195 | 2(1.03%) | 7(3.58%) | 5(2.55%) |  |  |  |
| 4 | Tashkent Region | 34 |  0 | 1(3.0%) | 1(3.0%) |  |  |  |
| In total | 309 | 6(1.94%) | 23(7.44) | 17(5.5%) | 0 | 1(0.32%) | 1(0.32%) |

Note: P<0.05 means the statistical significance as compared to values that characterize the group of donors exposed to HBV marker detection in lymphocyte content.

**CONCLUSIONS:**

A comparative analysis of HBV and HIV antigen detection rate, which was focused on blood serum and lymphocyte content obtained from 309 donors, allowed to establish the following:

1.In the event of EIA testing of blood serum from 309 donors for HBV infection detection, the test result was false-negative in 5.5% of cases. Use of *“A method for detecting lymphotropic viruses, such as HBV and HIV, in biological substrates having low concentration of viral particles, assessment of their viability, and exclusion of false-negative EIA testing results”* allowed to detect additionally HBV antigen in lymphocyte content of said 5.5% of donors.

2.In the event of EIA testing of blood serum for HIV infection detection, HIV antigen was detected in none (0%) of the donors being tested. Use of *“A method for detecting lymphotropic viruses, such as HBV and HIV, in biological substrates having low concentration of viral particles, assessment of their viability, and exclusion of false-negative EIA testing results”* during EIA testing of lymphocyte content for HIV infection detection, allowed to detect positive (!) result of EIA testing for HIV in 1(0,32%) donor.

3. *“A method for detecting lymphotropic viruses, such as HBV and HIV, in biological substrates having low concentration of viral particles, assessment of their viability, and exclusion of false-negative EIA testing results”* suggested by “New Medical Technologies” limited liability company enables the researchers to exclude false-negative EIA testing results when donor blood is tested to determine whether it is infected or not with HBV and HIV infection, which is essential to mitigation of the risk existing for donor blood recipients of their being infected via donor blood and blood-derived products in day-to-day health care.

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