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EVALUATION OF THE EFFECTIVENESS OF THE "DRY TUBE" METHOD USING THE PCR METHOD

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ABSTRACT

In recent years, HIV infection and Hepatitis C have been increasingly prevalent among various age groups in Uzbekistan. Consequently, accurate diagnosis of these diseases in laboratory settings, utilizing the most modern methods, has become essential. This article presents findings where samples from 49 HIV-positive and 26 Hepatitis C-positive patients were tested using the "Dry test tube" method, revealing that the sensitivity of the method was 74.8%.

Key words: polymerase chain reaction, "Dry test tube", Acquired Immune Deficiency Syndrome, Hepatitis C.

INTRODUCTION

In recent years, HIV infection and Hepatitis C are considered one of the most urgent medical and social problems both in the world and in Uzbekistan, and improving the diagnosis of these diseases remains a critical need [2,4,7].

It is well known that laboratory diagnosis of HIV infection began in the 80 years of the 20th century. Currently, diagnosis of HIV and Hepatitis C diseases is carried out using modern molecular genetic methods, with Enzyme Immunoassay

(EIA), Immunoblotting (IB) and Polymerase Chain Reaction (PCR) serving as examples [5,6].

4 generation test systems of EIA method for anti-HCV (Hepatitis C virus) detection have been developed, and the first-generation test system is not used due to low sensitivity. Hepatitis C virus can be detected by PCR 1-2 weeks after the patient is infected, while anti-HCV can be detected by EIA method in 80% of cases after 5-6 weeks of the disease and in 90% of patients after 12 weeks [1].

PCR is considered the most modern method of molecular genetic testing, offering high sensitivity and specificity. However, in addition to the advantages of this method, there are also certain limitations.

External quality control of laboratory tests is carried out through standardized panels. The main requirement for standardized panels is to obtain the same result in successive tests conducted in laboratories. This is done by stabilization (storage of samples) over a long period of time (1 year or more). Considering the storage period of genetic material (6 and 24 hours), the large size of the territory of Uzbekistan and the distance between the centers, we studied the effectiveness of a new alternative method, the “Dry test tube” technique [3].

The purpose of the research. To evaluate the effectiveness of the “Dry test tube” method using PCR.

Research materials and methods.

49 HIV-positive and 26 Hepatitis C-positive patients being treated in the 2nd and 5th departments of the Virology Institute under the Ministry of Health of the Republic of Uzbekistan, were selected for the study. All patients were initially tested by EIA and a positive result was obtained. Blood samples from 75 selected patients were collected by an alternative method, that is, by the “Dry test tube” method (1 ml of blood samples were taken from the wrist vein in a special test tube and dried in room conditions for at least 4 weeks). The samples were then tested using PCR, both with the “Dry Test Tube” method and the traditional method (fresh blood samples).

All tests were carried out in the Reference Laboratory of the Virological Research Institute.

For RNA extraction from plasma in PCR, we used the NucliSENSeasy MAG and “RIBOprep” test systems, and for amplification, the FRT PCR kit and Rotor-Gene 1.8.17.5 series analyzer were used.

Research results.

The majority of patients were aged between 15 and 60. There were 17 (22.7%) patients aged 15-25, 23 (30.7%) patients aged 25-40, and 35 (46.6%) patients aged 40-60 in the general group. Of these, 44 patients (58.7%) were male,

and 31 patients (41.3%) were female. In the first stage of scientific research, the blood plasma of HIV-infected patients was examined by conventional PCR and "Dry test tube" methods.

According to the test results, when we analyzed the viral load distribution, 15 people (20.5%, ±6.5) at the level of 10^3, 35 people at the level of 10^4 (46.1%, ±8.0), 19 people at the level of 10^5 (25.6%, ±3.8), and 6 patients at the level of 10^6 (7.7%, ±4.3) were detected by the conventional method. For the "dry test tube" method, the results were as follows: 50 people (66.6%, ±7.6) at the level of 10^3, 23 people at the at the level of 10^4 (30.7%, ±7.4), 2 people at at the level of 10^5 (2.5%, ± 2.5). No patients were detected at the level of 10^6. We can see the obtained results from the diagram below (Diagram 1).

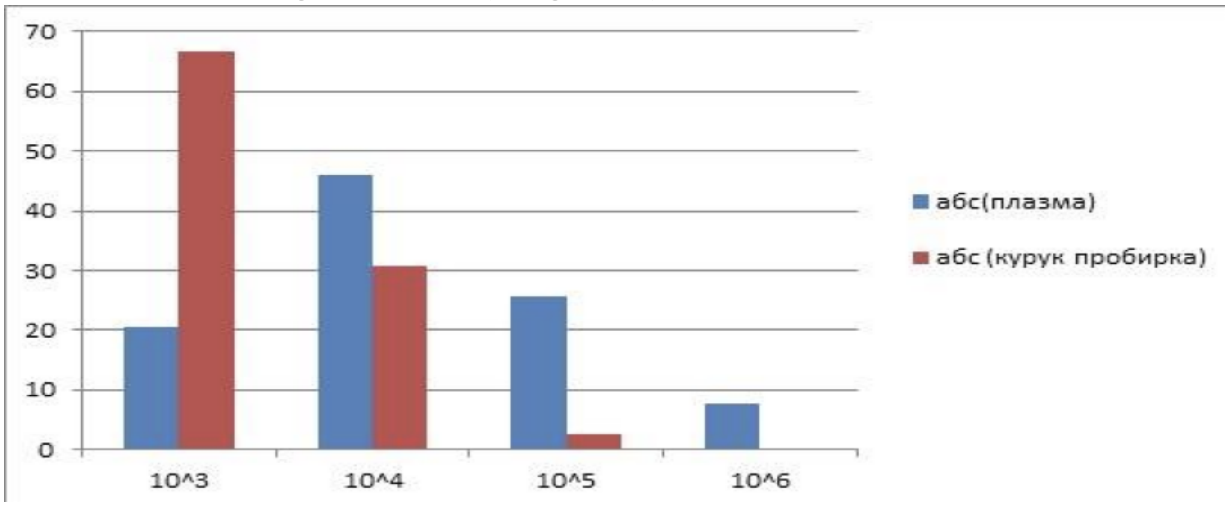

Diagram 1. Indicators of virus load in blood plasma of HIV-infected patients determined by conventional PCR and "dry test tube" method.

In the second stage of scientific research, the blood plasma of patients with Hepatitis C was examined by conventional and "dry test tube" PCR (table 1).

Table 1

Distribution of blood plasma of patients with hepatitis C according to the viral load determined by PCR using the conventional and "dry test tube" methods

Level of the viral load	abs (plasma)	%	± m	abs(dtt) +	%	± m
10^3	2	9,1	± 9,0	12	45,4	±15,7
10^4	10	36,3	±15,2	12	45,4	±15,7
10^5	7	27,2	±14,0	2	9,1	± 9,0
10^6	7	27,2	±14,0	-	-	±

As can be seen from Table 1, when we examined the blood plasma of 26 patients with Hepatitis C using both the traditional and “Dry test tube” methods and categorized the results based on viral load, the traditional method detected 2 patients (9.1%, ±9.0) at the 10^3 level, 10 patients (36.3%, ±15.2) at the 10^4 level, 7 patients (27.2%, ±14.0) at the 10^5 level, and 7 patients (27.2%, ±14.0) At the 10^6 level. In the “Dry test tube” method, 12 patients (45.4%, ±15.7) at the 10^3 level, 12 patients (45.4%, ±15.7) at the 10^4 level, 2 patients (9.1%, ±9.0) at the 10^5 level were detected. The 10^6 level was not detected in any of the patients.

The obtained results are also displayed in the diagram below (Diagram 2).

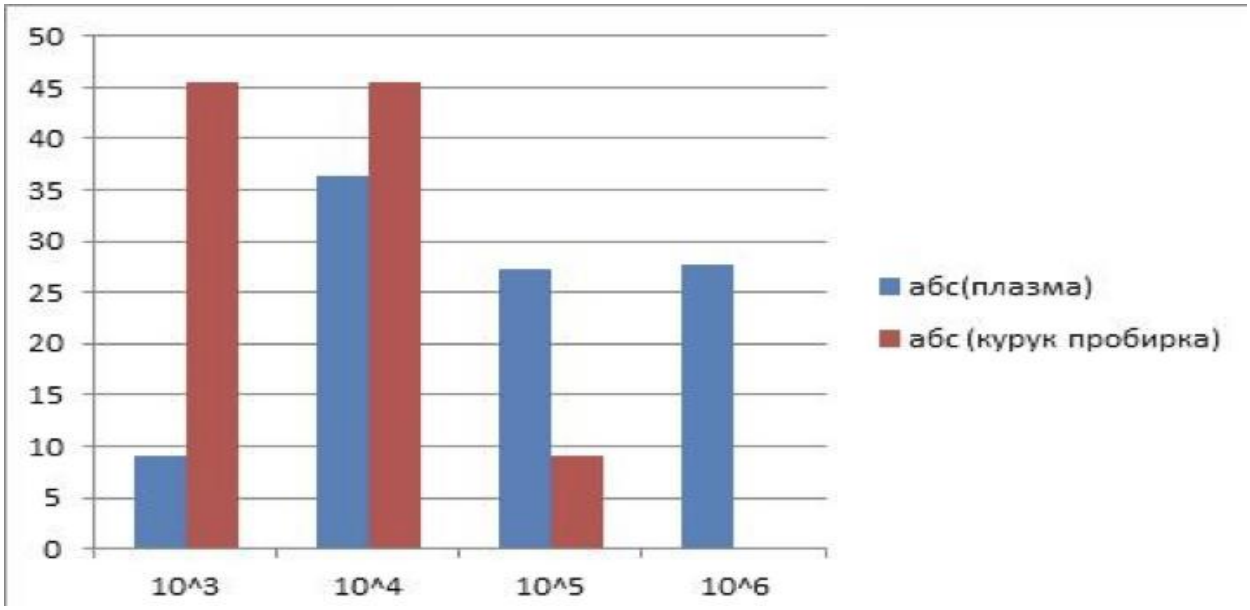

Diagram 2. Viral load indicators detected by PCR in the blood plasma of patients with Hepatitis C, using both the traditional method and the “Dry test tube” method.

When we analyzed the table and diagram above, the viral load detected by the traditional method was also detected by our alternative method. However, the “Dry test tube” method did not detect a viral load at the 10^6 level. It is evident that the viral load identified in plasma by the traditional method closely matched the viral load detected by the “Dry test tube” method in this research.

Conclusion.

1. When analyzing plasma samples from 75 patients with HIV infection and Hepatitis C, where the viral load exceeded 1000 copies of the virus per ml, the sensitivity of the “Dry test tube” method was found to be 74.8%.

2. The sensitivity of the blood samples (plasma) collected using the traditional method was determined to be 99.1%.

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