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COMPARATIVE STUDY OF HIV-POSITIVE HUMAN SERA WITH THIRD- AND FOURTH-GENERATION ENZYME IMMUNOASSAY TEST SYSTEMS

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The purpose of the work was to study the ability of test systems DIA-HIV and DIA-HIV-Ag/Ab produced by PJSC "SPC "Diaproph-Med" to detect HIV infection. Reference blood serum of HIV-positive patients was tested using immunoassay analysis. It was shown that early detection of HIV infection is more effective using test kits of the fourth generation, which should be used for primary screening for HIV, and third-generation test systems should be used to confirm the positive results of primary analysis.

Key words: *IEA*, early diagnosis of HIV, enzyme immunoassay test systems.

The HIV-infection/AIDS is today one of the most acute social and medical problems in Ukraine. Transmission rates of HIV-infection/AIDS in Ukraine are among the highest in Europe [1], and preventive measures have long been a state-level priority here. The reasons for early diagnosis of HIV infections are effective epidemiologic survey, timely prophylaxis, and if needed, short cycles of antiretroviral therapy in order to alleviate the course of disease [2, 3].

After HIV-infection, the blood serum markers of infection appear as follows: viral RNA, p24 antigen and antibodies to HIV [3, 4, 5]. The exact moment when HIV RNA, p24 antigen or antibodies can be detected depends on screening methods and individual reaction of the tested organism. Viral RNA can be revealed with PCR methods at the very beginning of disease. The antigen is found in two weeks after infection. The antibodies in blood of infected person are found after a "seroconversion window" period. Usually, the first antibodies, circulating during viremia, are immunoglobulins M (IgM) to gag gene proteins. In five to seven days after IgM antibodies, the infected organism starts synthesizing IgG antibodies. The most immunogenic HIV proteins are envelope glycoprotein gp160, surface glycoprotein gp120 and transmembrane protein gp41 env gene. Antibodies to these proteins are found in 98% of infected, and are more stable than antibodies to other antigens. Antibodies to the main structural core proteins of HIV-1 (p18 and p24 gag gene) are detected in nearly 75% of infected and less than in 50% of sick people with AIDS symptoms. The dynamics of antibody production in HIV-infection is connected to the clinical stages of disease. In almost two thirds of HIV-infected patients, antibodies to p18, p24 and p55 proteins develop as early as at the third to sixth weeks after infection. They are followed by antibodies to pol gene (p51, p66, p11, p34) and env products (gp160, gp120, gp41). In time, levels of antibodies to protein p24 and other products of gag gene decrease which correlates to the secondary antigenemia and general progress of disease [3, 5–7].

Analysis of HIV cases with exact date of infection (for example, due to blood transfusion) revealed that seroconversion can happen in three to 16 weeks. Reports of long seronegative periods in HIV-infected people are not confirmed in recent studies [2, 5, 7].

Creation of fundamentally new conjugates such as enzyme-tagged recombinant antigens allowed detecting IgA, IgM and IgG antibodies in third-generation test systems. With these systems, "seroconversion window" period can be reduced to 20 days in average [7–9].

Fourth-generation test systems, so called combined tests, are intended for simultaneous detection antibodies to HIV and p24 antigen HIV-1 [5]. Those systems allow detecting HIV infection markers earlier by four to eight days than with third-generation test systems. ELISA fourth-generation tests reduce the "seroconversion window" by one to two weeks in average [4, 6].

In "Diaproph-Med" (Ukraine), DIA-HIV-1/2 and DIA-HIVAg/Ab ELISA test systems for detection of HIV infection were developed. Their diagnostic characteristics (sensitivity and specificity) allow to implement them for screening and confirmation of positive results. Test systems are licensed by State and have the CE (Conformité Européenne) marking according to EU requirements.

Materials and Methods

Test-systems:

ELISA third-generation test system DIA-HIV 1/2 by Private Join-Stock Company NVK "Diaproph-Med". The test system is constructed as a one-stage "sandwich". Recombinant polypeptides Env-1 (gp120, gp41), Env-2 (gp36) and gag-1 (p17, p24) analogous to HIV-1 and HIV-2 antigens are sorbed in the solid phase. The conjugate contains a mixture of peroxidase recombinant polypeptides (gp120, gp41, gp36) analogous to HIV-1 and HIV-2 antigens and corresponding to total antibodies to HIV 1/2.

ELISA fourth-generation test system DIA-HIV-Ag/Ab by Private Join-Stock Company NVK "Diaproph-Med". The test system is constructed as two-stage double "sandwich" using biotin-streptavidin enhancement of the specific signal. Monoclonal antibodies to p24 HIV-1 (by Private Join-Stock Company NVK "Diaproph-Med"), diagnostic to viral antigen, and a mixture of Env-1 (gp120, gp41) and Env-2 (gp36) recombinant polypeptide analogues of HIV-1 HIV-2 antigens are sorbed on solid phase. Conjugate 1 contains a mixture of biotin-labeled synthetic polypeptides gp41 and gp36, analogous to HIV-1 and HIV-2 antigens. Immune complexes are determined by streptavidin conjugate (conjugate 2).

ELISA was performed according to the Instruction for those test systems. Results of ELISA were considered at ratio of optical density (OD) to the cut-off value (CO). Optical density was measured at 450/620 nm on Multiskan EX reader, Labsystems. Serum was considered positive at $OD/CO \geq 1.0$ or negative at $OD/CO < 1.0$.

Each serum sample was studied in three replicates; average value and standard deviation were calculated. Significance of difference in results of testing the studied serum in duplicates was determined by Student test for 95% confidence level [8].

Sera:

– SeraCare World-wide HIV-1 Performance panel WWRB302 with samples of sera with antibodies to various subtypes of HIV-1;

– SeraCare seroconversion panels — PRB 948, PRB 965, PRB 966;

– ZeptoMetrix seroconversion panels — 6243, 6244, 6248, 9012, 9016, 12007.

Results and Discussion

The ability of test systems DIA-HIV 1/2 and DIA-HIVAg/Ab to reveal infection of various subtypes of HIV was studied on 22 samples of SeraCare World-wide HIV-1 Performance panel WWRB302 panel. Nineteen samples contained antibodies to various subtypes of HIV-1 (No. 2–6, 8, 9, 12–23), two samples contained antibodies to HIV-2 (No. 7, 11) and one sample did not contain antibodies to HIV (Table 1).

Test systems DIA-HIV 1/2 and DIA-HIV-Ag/Ab detected all different subtypes of HIV-1 and HIV-2 samples as positive (100% sensitivity), sample No. 10 was found to be negative.

The ability of DIA-HIV 1/2 and DIA-HIV-Ag/Ab test systems for detection of early seroconversion was studied on nine commercial HIV-1 SeraCare and ZeptoMetrix panels. Table 2 presents the test results for panels SeraCare PRB 948, PRB 965 and PRB 966.

According to the passport data of panel PRB 948, HIV-specific antibodies and p24 HIV-1 antigen were absent in samples No. 1, 2, and 3. These sera tested negative in test systems DIA-HIV 1/2 and DIA-HIV-Ag/Ab. Sample No.4 with p24 HIV-1 antigen was positive according to Genetic Systems HIV-1 Ag EIA (Bio-RAD) and DIA-HIV-Ag/Ab. HIV in panel PRB 965 was revealed by test system Elecsys HIV Ag (Roche) earlier by seven days (sample No.2). HIV was not found with western blot in samples of panel PRB 966, only one sample (No.6, blood donation at 21st day) was positive in panel PRB 965, and for panel PRB 948 no data were available.

The next step was to study the ability of test kits DIA- HIV 1/2 and DIA-HIV-Ag/Ab to reveal early seroconversion on ZeptoMetrix panels, which contained samples taken at 0 to 25th days after infection (Table 3).

According to passport data, Genetic Systems HIV-1 Ag EIA (Bio-RAD) test system

Table 1. **Study results of WWRB302 serum panel in test systems DIA-HIV 1/2 and DIA-HIV-Ag/Ab**

Sample No.	Sample characteristics	DIA-HIV 1/2	DIA- HIV- Ag/Ab
		OD/CO	
2	HIV-1 subtype A	14.60±0.45	19.43±0.59
3	HIV-1 subtype G	27.92±0.63	27.84±0.63
4	HIV-1 subtype G	13.54±0.42	26.22±0.62
5	HIV-1 subtype A	21.95±0.58	28.93±0.65
6	HIV-1 subtype G	22.24±0.59	27.15±0.64
7	HIV-2	8.42±0.28	27.81±0.64
8	HIV-1 subtype G	17.15±0.56	29.21±0.65
9	HIV-1 subtype A	13.78±0.43	27.11±0.64
10	negative	0.61±0.05	0.56±0.05
11	HIV-2	4.32±0.17	26.93±0.62
12	HIV-1 subtype C	18.85±0.57	27.04±0.64
13	HIV-1 subtype A	14.62±0.45	28.02±0.65
14	HIV-1 subtype D	23.71±0.61	16.86±0.52
15	HIV-1 subtype D	22.65±0.6	26.88±0.62
16	HIV-1 subtype D	28.98±0.69	25.23±0.61
17	HIV-1 subtype D	27.70±0.64	27.96±0.64
18	HIV-1 subtype C	26.64±0.62	26.71±0.62
19	HIV-1 subtype C	20.81±0.58	2.04±0.11
20	HIV-1 subtype C	22.59±0.6	7.62±0.22
21	HIV-1 subtype B′	23.65±0.61	28.22±0.65
22	HIV-1 subtype E	22.22±0.59	14.06±0.45
23	HIV-1 subtype E	18.06±0.57	28.49±0.65

Note: Test results are given as OD/CO ratio values.

finds p24 antigen in seroconversion panel 6248 from the 18th day (sample No. 6), in seroconversion panel 9012 from the 16th day (sample No. 6). Test system DIA-HIV-Ag/Ab recognized these samples as positive. Test system DIA HIV-1/2 confirmed samples No. 7 in panel 6248 and No. 7 and 8 in panel 9012 as positive.

Testing kits on seroconversion panels of samples collected at 0 to 35 day after infection (ZeptoMetrix 6243, 6244, 9016) is showed in Table 4.

According to the passport data of panel ZeptoMetrix 6243, Genetic Systems HIV-1 Ag EIA (Bio-RAD) finds p24 HIV-1 antigen starting from 25st day after infection (sample No. 7), same as DIA-HIV-Ag/Ab. Test system DIA HIV-1/2 found positive samples in this panel starting from 33-rd day after infection (sample № 9), that is later by eight days. In panel ZeptoMetrix 6244, test systems Genetic Systems HIV-1 Ag EIA (Bio-RAD) and DIA-HIV-Ag/Ab identified HIV similarly from 28th day (sample No. 13). In panel 6244, DIA HIV-1/2 identified positive samples starting from 33-rd day (sample No. 14) unlike western blot which did not find HIV in any samples. Western blot (WB) is used to confirm HIV antibodies. It is not, however, used for screening because it is not without flaws, namely it is inconclusive for serum samples of patients at seroconversion window period. Lately the number of comparative studies on WB and ELISA has increased. Using ELISA to confirm

Table 2. **Study results of SeraCare seroconversion serum panels by test systems DIA-HIV 1/2 and DIA-HIV-Ag/Ab**

Panel/ Sample No.	Blood donation day	Genetic Systems HIV-1 Ag EIA (Bio-RAD)	Elecsys HIV Ag (Roche)	DIA-HIV-1/2	DIA-HIV-Ag/Ab	HIV-Blot2.2.(MP Biomedicals Asia Pacific Pte. Ltd.)
		ELISA results, OD/CV				Bands
PRB948 (1–3)	0–20	n.d.	n.a.	n.d.	n.d.	n.a.
PRB948 (4)	23	+	n.a.	n.d.	+	n.a.
PRB965 (1)	0	n.d.	n.d.	n.d.	n.d.	negative
PRB965 (2)	5	n.d.	+	n.d.	n.d.	negative
PRB965 (3)	7	n.d.	+	n.d.	n.d.	negative
PRB965 (4–5)	12–14	n.d.	+	+	+	not determined (gp160)
PRB965(6)	21	n.d.	n.d.	+	+	positive (gp160/gp120, p24)
PRB 966 (1–7)	0–37	n.d.	n.a.	n.d.	n.d.	n.d.
PRB966 (8–10)	44–51	+	n.a.	+	+	n.d.

Hereinafter: Results for Genetic Systems HIV-1 Ag EIA (Bio-RAD), Elecsys HIV Ag (Roche), HIV-Blot2.2. (MP Biomedicals Asia Pacific Pte. Ltd.) test systems are from passport data; n.a. — not available, n.d. — not determined.

Table 3. **Study results of ZeptoMetrix 6248 and 9012 serum panels by test systems DIA-HIV 1/2 and DIA-HIV-Ag/Ab**

Panel/ Sample No.	Day of blood donation	Genetic Systems HIV-1 Ag EIA(Bio-RAD)	Elecsys HIV Ag (Roche)	DIA- HIV 1/2	DIA-HIV-Ag/Ab	Chiron RIBA HIV-1/2 SIA (Novartis)
		ELISA results, OD/CV				Bands
6248 (1–5)	0–14	n.d.	n.a.	n.d.	n.d.	negative
6248 (6)	18	+	n.a.	n.d.	+	negative
6248 (7)	25	+	n.a.	+	+	negative
9012 (1–5)	0–14	n.d.	n.a.	n.d.	n.d.	n.d.
9012 (6)	16	+	n.a.	n.d.	+	n.d.
9012 (7–8)	21–23	+	n.a.	+	+	n.d.

laboratory diagnosis of HIV significantly reduces the seroconversion window period due to high sensitivity of test systems to HIV 1 and 2 antigens and antibodies [4, 9].

Test results of panel ZeptoMetrix 12007 with samples on 0 to 133 day by test systems DIA- HIV 1/2 and DIA-HIV-Ag/Ab are presented in Table 5.

According to data in Table 5, p24 antigen was found by test systems starting from 117th day (sample No 4) by test systems Genetic Systems HIV-1 Ag EIA (Bio-RAD) and

Table 4. **Study results of ZeptoMetrix 6243, 6244, and 9016 serum panels by test systems DIA-HIV 1/2 and DIA-HIV-Ag/Ab**

Panel/ Sample No.	Day of blood donation	Genetic Systems HIV-1 Ag EIA(Bio-RAD)	Elecsys HIV Ag (Roche)	DIA- HIV 1/2	DIA-HIV-Ag/Ab	Chiron RIBA HIV-1/2 SIA (Novartis)
		ELISA results, OD/CO				Bands
6243 (1–6)	0–20	n.d.	n.a.	n.d.	n.d.	negative
6243 (7)	25	+	n.a.	n.d.	+	negative
6243 (8)	28	+	n.a.	n.d.	+	negative
6243 (9–10)	33–35	+	n.a.	+	+	negative
6244 (1–12)	0–26	n.d.	n.a.	n.d.	n.d.	negative
6244 (13)	28	+	n.a.	n.d.	+	negative
6244 (14–15)	33–35	+	n.a.	+	+	negative
9016 (1–8)	0–27	n.d.	n.a.	n.d.	n.d.	n.d.
9016 (9–10)	30–34	+	n.a.	n.d.	+	n.d.

Table 5. **Study results of ZeptoMetrix 12007 serum panel by test systems DIA -HIV 1/2 and DIA-HIV-Ag/Ab**

Panel/ Sample No.	Day of blood donation	Genetic Systems HIV-1 Ag EIA(Bio-RAD)	Elecsys HIV Ag (Roche)	DIA- HIV 1/2	DIA- HIV- Ag/ Ab	Chiron RIBA HIV-1/2 SIA (Novartis)
		ELISA results, OD/CO				Bands
12007 (1–3)	0–54	n.d.	n.a.	n.d.	n.d.	n.d.
12007 (4–5)	117–119	+	n.a.	n.d.	+	n.d.
12007 (6)	124	+	n.a.	+	+	positive (gp41, p24/26)
12007 (7–9)	126–133	n.d.	n.a.	+	+	positive (gp41, p24/26)

DIA-HIV-Ag/Ab. Antibodies were found starting from 124th day (sample No. 6) by western blot Chiron RIBA HIV-1/2 SIA (Novartis) and test kit DIA-HIV 1/2.

Thus, at least 21 samples out of 10 studied panels contain p24 HIV-1 antigen and 38 samples contain antibodies to HIV-1. Results of western blot for eight panels excepting PRB948 and WWRB302 were used in comparison. Five samples tested positive and four were inconclusive in western blot. According to passport data of Genetic Systems HIV-1 Ag EIA (Bio-RAD) test system, 21 samples were positive (data were available for nine panels). In those panels DIA-HIV-Ag/Ab test system detected 27 positive samples and DIA-HIV 1/2 test system found 17 positive samples. In total, in 10 panels DIA-HIV-Ag/Ab test system identified 48 positive samples (OD/CO $\geq$ 1.0) and DIA-HIV 1/2 test system identified 38 positive samples. Thus, DIA-HIV-Ag/Ab kit is significantly better in identifying early HIV infection.

Hence, comparative analysis of diagnostic sensitivity of DIA-HIV 1/2 and DIA-HIV-Ag/Ab test systems ("SPC Diaproph-Med") revealed that the problem of early HIV diagnostics can be solved by implementation highly sensitive complex tests for p24 HIV antigen and antibodies in laboratory practice [3]. Antigen p24 is associated with viral replication and is found in blood in two weeks after infection, preceding antibodies to HIV antigens by one-two weeks. Study of 10 seroconversion panels showed that identification of early HIV infection was more effective using fourth-generation test systems. These systems should be used in primary screening for HIV, and third-generation test systems should be used to confirm results of primary screening. High sensitivity of DIA-HIV 1/2 and DIA-HIV-Ag/Ab test systems allows implementing them in laboratory diagnostics of HIV infections.

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ПОРІВНЯЛЬНИЙ АНАЛІЗ СИРОВАТОК ВІЛ-ІНФІКОВАНИХ В ІМУНОЕНЗИМНИХ ТЕСТ-СИСТЕМАХ 3-ГО ТА 4-ГО ПОКОЛІННЯ

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Метою роботи було дослідити здатність тест-систем DIA-HIV 1/2 та DIA-HIV-Ag/Ab виробництва ПрАТ «НВК «Діапроф-Мед» виявляти ВІЛ-інфекцію. Перевірку еталонних сироваток крові хворих на ВІЛ проводили методом імуноензимного аналізу. Показано, що виявлення ранньої ВІЛ-інфекції ефективніше за допомогою тест-систем 4-го покоління, які доцільно використовувати при первинних дослідженнях на ВІЛ, а тест-системи 3-го покоління — для підтвердження позитивних результатів первинного аналізу.

Ключові слова: ІЕА, рання діагностика ВІЛ, імуноензимні тест-системи.

СРАВНИТЕЛЬНЫЙ АНАЛИЗ СЫВОРОТОК ВИЧ-ИНФИЦИРОВАННЫХ В ИММУНОЭНЗИМНЫХ ТЕСТ-СИСТЕМАХ 3-ГО И 4-ГО ПОКОЛЕНИЯ

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Целью работы было исследование способности тест-систем DIA-HIV 1/2 и DIA-HIV-Ag/Ab производства ЧАО «НВК «Диапроф-Мед» выявлять ВИЧ-инфекцию. Проверку эталонных сывороток крови больных ВИЧ проводили методом иммуноэнзимного анализа. Показано, что выявление ранней ВИЧ-инфекции более эффективно с помощью тест-систем 4-го поколения, которые целесообразно использовать при первичных исследованиях ВИЧ, а тест-системы 3-го поколения — для подтверждения положительных результатов первичного анализа.

Ключевые слова: ИЭА, ранняя диагностика ВИЧ, иммуноэнзимные тест-системы.