Significance of detection of p24 antigen to reduce the time of HIV diagnosis in case of indeterminate and negative results of immunoblotting assay

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Abstract

Introduction. The main method of laboratory diagnostics of HIV infection is simultaneous detection of antibodies and HIV p24 antigen by enzyme immunoassay. Positive EIA results are confirmed using Western Blot (WB). Interpretation of negative or indeterminate WB results is one of the main problems of HIV laboratory diagnostics. The additional study for HIV p24 detection or PCR is recommended in this case. The sensitivity of assay for HIV p24 detection is very crucial because the concentration of HIV p24 is very low at the earliest stages of HIV infection and indeterminate or negative WB results mostly occur during seroconversion window period.

Objectives. Evaluate the significance of detection of p24 antigen in the samples, which are positive in HIV screening EIA, but negative or indeterminate in WB.

Methods. Four assays were used in this study. "DS-EIA-HIV-AgAb-SCREEN" is a 4th generation assay (RPC "Diagnostic Systems"), "DS-EIA-HIV-Ag-SCREEN" (RPC "Diagnostic Systems") is an enzyme immunoassay intended for detection of HIV-1 p24 antigen with sensitivity 0.025 IU/ml. "Genetic Systems HIV-1 Ag EIA" (BioRad, France) is an enzyme immunoassay intended for detection of HIV-1 p24 antigen with sensitivity 0.4 IU/ml; and New Lav Blot I (BioRad, France). The sensitivity of the kits "DS-EIA-HIV-Ag-SCREEN" and "Genetic Systems HIV-1 Ag EIA" was evaluated using "HIV-1 p24 ANTIGEN 1st international r eference reagent", NIBSC Code 90/636. All used assays are CE certified. Samples from HIV infected patients (n=904) were provided by Regional Center for prevention and control of AIDS and infection diseases, Nizhny Novgorod (Russia).

Results. The HIV positive in EIA and negative/indeterminate in WB samples (n=904) were assessed for presence of p24 antigen. The highly sensitive test "DS-EIA-HIV-Ag-SCREEN" detected and confirmed p24 antigen in 209 samples(23.11%). 59 samples of them (28.23%) were negative in WB, and 150 samples (71.77%) gave indeterminate result in WB. According to the results of evaluation of HIV positive samples with the kit "Genetic Systems HIV-1 Ag EIA", p24 antigen was detected in 193 samples (21.34%). Antigen p24 was not detected in 16 samples by kit "Genetic Systems HIV-1 Ag EIA", due to lower sensitivity at detection of p24 antigen. Out of 193 samples containing p24 antigen, 58 samples (30.05%) were negative in WB, and 135 samples (69.95%) gave indeterminate result in WB.

Conclusion. High percentage of HIV p24 positive samples among HIV positive samples defined as indeterminate or negative in WB, confirms the significance of HIV p24 detection at HIV diagnostics. The applying of HIV p24 detection allows to reduce significantly the number of WB indeterminate or even negative results. The use of more sensitive HIV p24 assay increases detectability of HIV infection and this allows to initiate treatment and anti-epidemic measures in due time, as well as to minimize the disease transmission.

Introduction

AgAb Combo tests intended for detection of HIV are of great value at early stages of infection due to their capability to detect p24 antigen. The improvement of the manufacturing technology of EIA tests allows to reach high sensitivity (up to 99.99%), meanwhile the immunoblotting sensitivity is 97%. That is why negative or indeterminate result in WB combined with positive EIA result may indicate an early seroconversion period, which is characterised by low level of specific antibodies. Antibodies may appear at early stages of infection, however their concentration may be below the detection limit of applied method.

The problem of early HIV diagnostics in case of negative or indeterminate result in WB may be solved by additional testing for p24 antigen. However in this case the sensitivity of test is critical. The early detection of infection due to analysis for p24 antigen contributes in reducing the HIV diagnostic period and prompt medical consultation of HIV infected individuals; as well as it allows to carry out preventive measures.

Aim

Evaluate the significance of detection of p24 antigen in the samples, which are positive in HIV screening EIA, but negative or indeterminate in WB.

Materials and methods

Samples from HIV infected patients were screened by the 4th generation assay "DS-EIA-HIV-AgAb-SCREEN" (RPC "Diagnostic Systems") with the sensitivity 0.5 IU/ml at detection of HIV-1 p24 antigen. The positive results were confirmed by New Lav Blot (BioRad, France). Serum samples from 904 patients, which were positive in EIA, but negative or indeterminate in WB, were included in the study. All samples (n=904), which gave positive results in EIA and indeterminate or negative results in WB, were additionally tested for p24 antigen with EIA "DS-EIA-HIV-Ag-SCREEN"(RPC "Diagnostic Systems") and EIA "Genetic Systems HIV- 1 Ag EIA"(BioRad, France). The sensitivity of the used kits "DS-EIA-HIV-Ag-SCREEN" and "Genetic Systems HIV-1 Ag EIA" was evaluated using "HIV-1 p24 ANTIGEN 1st international reference reagent", NIBSC Code 90/636 (table 1). All p24 antigen positive samples were confirmed by the neutralization method.

Results

Out of 904 samples, which were positive in EIA and negative or indeterminate in WB, the highly sensitive kit "DS-EIA-HIV-Ag-SCREEN" (sensitivity 0.025 IU/ml) detected and confirmed p24 antigen in 209 samples (23.11%). 59 (28.22%) samples of them were negative in WB, and 150 (71.78%) samples were indeterminate in WB. According to the results of evaluation of 904 samples with the less sensitive kit "Genetic Systems HIV-1 Ag EIA" (sensitivity 0.4 IU/ml), p24 antigen was detected in 193 (21.34%) samples. 58 (30.05%) out of 193 p24 antigen positive samples had negative results in WB; and 135 (65,69%) samples had indeterminate result in WB. Fig.1. It is shown that detection of p24 antigen in the group of samples with WB indeterminate results, in average, was 2.5 times higher, than in the group of samples with WB negative result ($p<0.05$). Table 2. When using the more sensitive kit "DS-EIA-HIV-Ag-SCREEN" intended for detection of p24 HIV-1 antigen, 16 samples with early HIV infection were found additionally.

Analytical sensitivity of kits for detection of HIV infection

Table 1

Standard	Analytical sensitivity of p24		
	DS-EIA-HIV-Ag-SCREEN	Genetic Systems HIV- 1 Ag EIA	DS-EIA-HIV-AgAb-SCREEN
HIV 1 p24 antigen 1st international reference reagent" (NIBSC)	0.025 IU/ml	0.4 IU/ml	0.5 IU/ml

Detection of p24 antigen in groups of sera with WB indeterminate or negative results

Table 2

Kits	Number tested	Number p24 positive	Confirmed p24 antigen in the group with WB indeterminate / negative results *	
			indeterminate WB	negative WB
DS-EIA-HIV-Ag-SCREEN	904	209 (23,11%)	150	59
Genetic Systems HIV-1 Ag EIA	904	193 (21,34%)	135	58

Note - * significance level between WB indeterminate or negative results $p<0,05$

The prevalence of p24 antigen positivity among HIV positive samples in EIA, but negative or indeterminate in WB

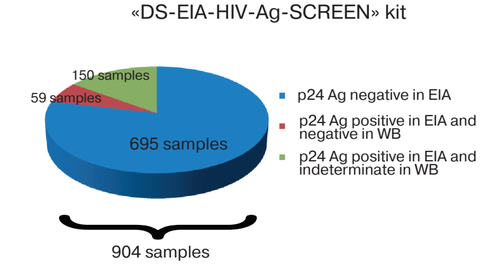

Fig.1

Conclusion

Additional analysis for p24 antigen of samples with positive result in EIA, but indeterminate or negative result in WB allows to identify cases of early seroconversion. The test "DS-EIA-HIV-Ag-SCREEN" may be used in the HIV confirmation algorithm in countries with limited laboratory capacities.

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