20th European Congress of Clinical Microbiology and Infectious Diseases
Vienna, Austria, 10-13 April 2010

Poster presentation
Viral hepatitis - seroepidemiology

Abstract: P1113

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Patients PCR status and hepatitis C avidity assay

S. Klimashevskaya, T. Ulanova, A. Burkov, H. Fields, A. Obriadina
(Nizhny Novgorod, RU; Atlanta, US; Saronno, IT)

Objectives: Antibody Avidity is serological marker of primary infection and avidity test is a reliable method to distinguish acute primary HCV infections and chronic HCV infections for certain. But during this study some limitations for the avidity assay were found.

Methods: 220 samples from 21 commercially available seroconversion panels, 480 samples from anti-HCV and HCV RNA-positive blood donors and samples from 21 anti-HCV positive, but HCV RNA negative blood donors with resolved infection were tested. The detection of antibody avidity was based on an indirect ELISA method using a mixture of antigens, containing epitopes to core-1b, NS3-1a, 1b and NS4 (the artificial mosaic protein contains the HCV NS4 immunodominant regions from 1, 2, 3, 5 genotypes).

Results: The mean AI value for seroconversion samples, obtained <65 days after the last anti-HCV negative result was 18.6% (95% CL, 3.5% to 33.7%). Samples from anti-HCV and HCV RNA positive patients with chronic HCV infection showed the mean AI value of 100% (95% CL, 83.1% to 116.9%). Samples from patients with resolved infection showed a mean AI of 54% (95% CL, 32.8% to 75%). The observed differences were significant ($P < 0.001$).
Patients with high PCR level had an AI increase during a shortest time than patients with low PCR Level. The best correlation between AI value and time after infection onset was observed from patients with PCR status more than 1,000,000 copies: $y = 0.83x + 1.5$ against $y = 0.3x + 31$ for patients with PCR status 10,000-100,000 copies or $p = 0.08x + 49$ for patients with PCR status <1,000 copies.

Conclusions: The AI may depends on patient PCR status. Persons with low PCR level or PCR negative (resolved infection) may have the low AI for a long time and the avidity assay is more reliable for PCR positive specimens with high PCR level.

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Diagnostics in virology

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Advantages of the state-of-the-art ELISA test at verification of an early HIV infection

I. Sharipova, N. Denisova, E. Baranova, M. Susekina, V. Puzyrev, K. Sarkisyan, M. Vorob'eva, A. Obriadina, A. Burkov, **T. Ulanova**

(Nizhny Novgorod, Moskow, RU)

Background: One of the problems of EIA diagnostic HIV infection associated with the reliable confirmation of the screening assays results. The basic difficulties are connected with confirmation screening result at early stage of seroconversion because immune blot assays (WB) are not intended for detection of presence p24 antigen - early serological a marker of an HIV infection. The other problem is "indeterminate" WB result. The aim of present study was to evaluate EIA test "DS-EIA-HIV-Ab/Ag-spectrum" as a supplemental assay for confirmation of the HIV positive results at early stage of seroconversion.

Materials: The "DS-EIA-HIV-Ab/Ag-spectrum" is an *in vitro* qualitative EIA for the detection of antibodies to individual proteins of HIV 1 (including HIV 1 group 0), HIV 2 and HIV-1 p24 antigen in human serum or plasma. Wells of microtiter plate are separately coated by recombinant proteins comprising diagnostic relevant epitopes of HIV 1 structural proteins gp41 and HIV-1 group 0 gp 41, gp120, p24, p31 and gp36 of HIV 2 and mouse monoclonal antibodies to HIV 1 p24. Sensitivity of the test was evaluated by 16 commercial available seroconversion panel (total n = 167) [ZeptoMetrix and BBI (USA)].

Results: EIA test "DS-EIA-HIV-Ab/Ag-spectrum" permits confirmation of earlier detection of HIV infection. This assay is able to confirm 100 out of 167 seroconversion specimens as HIV positive. 28 of them were positive for p24 antigen only. The delay of HIV infection detection by EIA test "DS-EIA-HIV-Ab/Ag-spectrum" in comparison with detection of HIV virus RNA is 1.2 days. WB can confirm only 13 samples out of 167 as HIV positive and 54 samples as indeterminate. The delay of HIV infection detection in comparison with detection of HIV virus RNA is 18-19 days.

Conclusion: The received results demonstrated high diagnostic efficiency of new supplemental assay. Opportunity of detection p24 antigen and high specificity allow to confirm screening results from early stages of HIV infection and reducing number indeterminate results received by WB.

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The highly sensitive enzyme immunoassay for HBsAg detection as the alternative method to nucleic acid testing

N. Egorova, S. Igolkina, I. Pyrenkova, I. Sharipova, V. Puzyrev, A. Obriadina, A. Burkov, **T. Ulanova**

(Nizhniy Novgorod, RU; Saronno, IT)

Background: Enhancement of sensitivity of the currently available EIA assays is very important be-cause the early stage of HBV infection when HBsAg level is below detection limit of the best available EIA kits (0.05-0.1 IU/ml) is one of the main reasons of transfusion-associated hepatitis A. The aim of the study was to evaluate the advantage of highly sensitive assay DS-EIA-HBsAg-0.01 (0.01 IU/ml Second Interna-tional Standard for HBsAg subtype adw2, genotype A, NIBSC code number: 00/588) for detection of HBsAg during seroconversion period.

Methods: The correlation between HBsAg and HBV DNA presence in seronversion samples has been studied. Twenty eight commercial panels PHM - 911, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935A(M), 935B (Boston Biomedica Inc.) and HBV - 6271, 6273, 6275, 6277, 6278, 6279, 6281, 11001, 11002, 11003, 11006, 11008, 11011, 11012, 11017 (ZeptoMetrix Corp.) were used. The aggregate score was calculated by summing the total number of reactive specimens in the all panels detected by each test. The mean number of days delay in detection of HBsAg and HBV DNA were also calculated.

Results: DS-EIA-HBsAg-0.01 assay detected 203 samples as HBsAg positive out of 283 seroconversion samples. Only 181 seronversion samples out of 283 were detected as HBV DNA positive.
The detection of HBsAg and HBV DNA ranged 0-133 days with the means delay in detection of HBsAg of 21.85 days and detection of HBV DNA of 24.48 days. DS-EIA-HBsAg-0.01 detected HBsAg in the specimens of the PHM (926, 931, 933), HBV (6277, 6279, 11001, 11012, 11017) panels by one bleed earlier and in the specimens of the panels PHM925, HBV11002, PHM932, HBV6275 by two, two, four and five bleeds, accordingly, earlier than the initial detection of HBV DNA (100-400 copies/ml) occurred.
With the use of the DS-EIA-HBsAg-0.01 for evaluation the specimens of the PHM (927, 928, 929, 930, 934, 935A(M)), HBV (6271, 6273, 6281, 11003, 11006, 11008, 11011) panels, the moment of detection of HBsAg coincided in the time of initial detection of HBV DNA. Hence using more highly sensitive assay allows determining HBsAg simultaneously to HBV DNA or even earlier.

Conclusion: Increasing sensitivity EIA for HBsAg detection up to a level comparable to sensitivity of nucleic acid testing (NAT) allows to consider it as the possible alternative to other methods which will raise quality of screening of donor blood, will allow to reduce the risk of posttransfusional hepatitis B infection.

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The evaluation of the ELISA kit "EIA-anti-HCV" with new recombinant antigens

G.B. Bochkova, V. Puzyrev, A. Obriadina, A.N. Burkov, T. Ulanova
(Nizhniy Novgorod, RU)

Introduction: High heterogeneity is typical for hepatitis C virus. The new generation enzyme immunoassay "EIA-ANTI-HCV" intended for the detection of antibodies to viral hepatitis C in human serum or plasma was developed. The recombinant antigens comprising only diagnostically relevant regions of different variants of native HCV proteins were specially selected.

Aim: The evaluation of the ELISA kit "EIA-anti-HCV".

Objectives and Methods: The various sequences of recombinant antigens comprising HCV Core, NS3, NS4, NS5 were adsorbed on the microtiter plate. Diagnostic value of the assay was studied by testing 1004 anti-HCV positive samples of patients with confirmed hepatitis C diagnosis, including 338 samples with determined genotype 1-6 (76 samples of genotype 1, 51 sample of genotype 2, 38 samples of genotype 3, 25 samples of genotype 4, 5 samples of genotype 5, 8 samples of genotype 6); samples of 31 commercial seroconversion panels (BBI Inc., ZeptoMetrix), samples of the "Anti-HCV Mixed Titer Performance Panel BBI PHV 206" (BBI Inc.) Sera samples of patients with acute (n = 30) and chronic (n = 439) hepatitis C were studied for the clinical efficiency assessment.
Diagnostic specificity was studied by testing samples of healthy donor blood (n = 8107), clinical patients (n = 1225), pregnant women (n = 735), patients with hepatitis B (n = 600).

Results: Out of 259 samples from 31 tested seroconversion panels the kit "EIA-ANTI-HCV" detected 101 samples (40%) as positive. The kit detected 23 positive results and indicated 2 negative results according to data available from insert package of the panel "Anti-HCV Mixed Titer Performance Panel BBI PHV 206". It should be mentioned that the value OD/Cut-off of the most of positive results were higher than the passport date. The diagnostic sensitivity of the kit "EIA-ANTI-HCV" at testing anti-HCV positive samples with clinical diagnosis of acute and chronic hepatitis C was 100%. Sensitivity of the kit during testing the samples with different genotypes was 100%.
The study showed high specificity of the kit "EIA-ANTI-HCV".

Conclusion: The received results demonstrated high diagnostic efficiency of ELISA kit in the combination with high specificity.