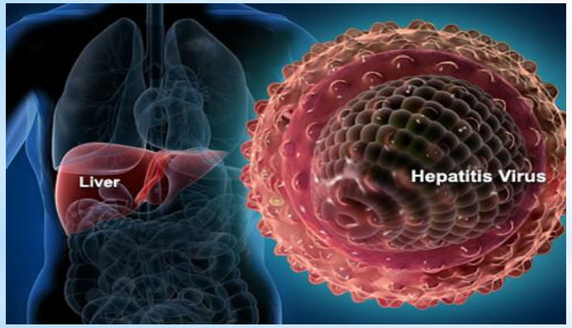
Improved detection of hepatitis B virus surface antigen by new enzyme immunoassay

E.S. Kochina[1], I.U. Pyrenkova[1] , Y.S. Ptitsyna*[1], N.I. Egorova[1], E.U. Maiorova[1], S. Biallas[2], P. Roth[2], A.N. Burkov [1] , A.P. Obriadina [1]

[1] RPC “Diagnostic Systems”, Nizhniy Novgorod, Russia, http://www.npods.ru, phone: (831) 434 86 83, Fax: (831) 434 86 83

[2]AB Diagnostic Systems GmbH, Berlin, Germany, http://www.ab-ds.de, phone: +49 30 208 987 160 /+49 30 208 987 199, e-mail: info@ab-ds.de.

Background: Hepatitis B virus (HBV) infection is a major cause of acute and chronic hepatitis, and of its long-term complications. It is the most variable among DNA viruses, mostly because of its unique life cycle which includes the activity of error-prone enzyme, reverse transcriptase, and the very high virion production per day [I.Lazarevic et al., 2014].



The use of highly sensitive test for HBsAg detection allows reducing the period of serological window. It will enhance quality of hepatitis B diagnostics. Detection of the most important HBsAg mutations is also necessary.

Aim: In this study a new “sandwich” solid-phase enzyme immunoassay abia HBsAg was evaluated and compared to a well-established test.

Material/methods: The kit under evaluation was abia HBsAg, cat. #DK.013.01.3 (AB Diagnostic Systems GmbH, Germany). Murex HBsAg Version 3, cat. #9F80-05 (Murex Biotech Limited, UK) was used as a reference assay.

38 seroconversion panels were investigated (ZeptoMetrix Corporation – 33 panels (HBV 6271, 6272, 6275, 6276, 6285, 6288, 6289, 6293, 6290, 6292, 9073, 11000, 11002, 9074, 11006, 11007, 11011, 11008, 11009, 11010, 11012, 11016, 11013,11028, 11017, 11024, 11026, 11027, 11029, 11031, 11052, 11056, 11069) and BBI Diagnostics – 5 (PHM 906, 918, 921, 924, 936)).

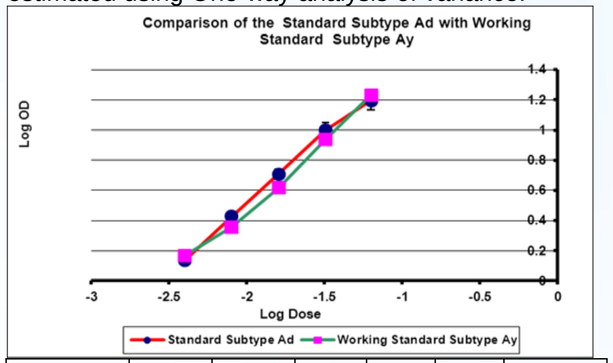
WHO 3rd International Standard for HBsAg (NIBSC Code:12/226), Standard for HBsAg, Subtype Ad, 100 U/ml (Paul-Ehrlich-Institut) and Working standard HBsAg subtype Ay, 50 000 U/ml (Paul-Ehrlich-Institut) were used for determination of analytical sensitivity.

Native mutant panel cat. #DH1200, (Trina Bioreactives AG, Germany) was utilized to evaluate mutant detection by the abia HBsAg. For specificity assessment 5008 samples from unselected donors were tested by the abia HBsAg (Blood donor service from German Red Cross, Frankfurt/Main, Germany).

Results: The analytical sensitivity was evaluated with WHO 3rd International Standard for HBsAg (NIBSC Code: 12/226) and defined at 0.02 IU/ml. It was calculated as limit of detection in accordance with the CLSI document EP17-A2 with a probit regression model.

The abia HBsAg detects both Ad and Ay HBsAg subtypes as positive. The minimal HBsAg concentration detected by the abia HBsAg, (A=0.120) was 0.008 U/ml, according to Standard for HBsAg, Subtype Ad, 100 U/ml (Paul-Ehrlich-Institut) and 0.004 U/ml according to Working standard HBsAg subtype Ay (Paul-Ehrlich-Institut). Thus, the abia HBsAg demonstrated comparable ability to detect Ad and Ay subtypes. The determination of parallelism and linearity between sets of dose-response data (results of Ad and Ay serial dilution testing) is an integral part of the similarity indication of the substances.

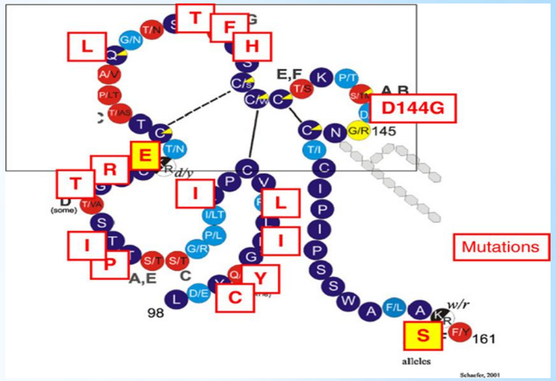
Parallelism and linearity between Standard Subtype Ad and Working Standard Subtype Ay dilutions were estimated using One-way analysis of variance.



Variation source	Degree of freedom	Sum of squares	Aver-age	F-theory	F-prac-tice	Results
Measure-ment (dose)	9	5.8682	0.6520	2.21	1576.7	significantly
Preparations of Standards	1	0.0095	0.0095	4.17	23.0	significantly
Linear regression	1	5.8212	5.8212	4.17	14076.9	significantly
Parallelism	1	0.0001	0.0001	4.17	0.2	Yes
Linearity	6	0.0375	0.0062	2.42	15.1	No
The residual error	30	0.0124	0.0004			
Total	39	5.8806				

Seroconversion sensitivity of abia HBsAg and Murex HBsAg Version 3 was evaluated. In this study the abia HBsAg detected more panel members (191 of 477) than Murex HBsAg Version 3 (171 of 477).

The native mutant panel (n=48) was tested in the abia HBsAg. The panel includes serum samples with different HBV genotypes, HBsAg subtypes and serotypes: A1 (ayw1), A2 (adw2), A3 (ayw1), B4 (ayw1), C (adr), D1 (ayw1 and ayw2), D2 (ayw2 and ayw3), D3 (ayw3), D4 (ayw2), D7 (ayw2), EE (ayw3 and ayw4).



Samples from this panel represent wide variety of HBsAg mutants:

1) amino acid substitutions at positions 116, 118, 120, 123, 126, 124, 129, 130, 133, 134, 143, 144, 145; 2) insertions at amino acid position 116 and 114/115; 3) mutation W172-stop outside of main hydrophilic region. All samples were tested undiluted and diluted 1:10 in human negative plasma. All samples with mutation in the first and second loops were identified as positive by the abia HBsAg.

Total specificity of the abia HBsAg with 5008 blood donor specimens was 99.86%.

Conclusions: The abia HBsAg permits earlier HBsAg detection than the reference assay. Diagnostic capacity of the studied test is broad enough. High sensitivity of abia HBsAg does not impair the specificity of the assay.