MULTICENTER STUDY OF THE NEW HIGHLY SENSITIVE AND SPECIFIC ENZYGNOST® HBsAg 6.0 ASSAY

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Background: Highly sensitive hepatitis B surface antigen (HBsAg) screening assays are crucial for efficiently avoiding transfusion-associated HBV infections. At the same time high specificity is required to reduce retesting and supplementary testing efforts, to avoid unnecessary blood destruction and thus to make routine blood donation screening cost effective. The new Enzygnost® HBsAg 6.0 assay from Siemens Healthcare Diagnostics was designed to comply with these continuously rising requirements.

Aims: To determine the sensitivity and specificity performance data of Enzygnost® HBsAg 6.0 a European multicenter study was conducted at 13 study sites. Sensitivity results were compared with the former assay version Enzygnost® HBsAg 5.0 and in addition with up to 27 CE-marked HBsAg assays.

Methods: Sensitivity performance was determined at seven study sites by evaluating: (i) diagnostic sensitivity using a total of 1284 samples; (ii) 37 commercially available seroconversion panels in comparison to 27 CE-marked assays; (iii) analytical sensitivity with Paul Ehrlich Institute (PEI) standard for HBsAg subtypes ad (1,000 PEI-U/ml) and ay (50,000 PEI-U/ml), French national reference HBsAg panel (“Ag HBs” SFTS/2005), WHO 2nd international standard (00/588), HBsAg ad/ay sensitivity panel PHA808 (Seracare) and the future WHO HBV genotype reference panel consisting of 16 samples with biochemically determined HBsAg concentrations comprising the HBV genotypes A to H, as developed by the German National Consultant Laboratory in collaboration with the WHO and the PEI. For the PEI and WHO standards comparison data from 17 CE-marked assays were available.

Specificity performance was determined at eight study sites by evaluating: (i) unselected serum (n=31,221) and plasma (n=20,313) samples from blood donors as well as (ii) potentially cross-reacting samples (n=784).

Results: Evaluation of the diagnostic sensitivity revealed 100% and 99.8% for Enzygnost® HBsAg 6.0 and Enzygnost® HBsAg 5.0, respectively. Three samples which were from chronic HBV patients in the resolution phase were negative with Enzygnost® HBsAg 5.0 but showed weak reactivity in Enzygnost® HBsAg 6.0, which could be confirmed by neutralization assay.
Early detection of HBV infection as evaluated by seroconversion panels was improved for Enzygnost® HBsAg 6.0 by 2.51 days compared to Enzygnost® HBsAg 5.0 and by 0.32 days compared to the current most sensitive CE-marked assay included in the comparison. The least sensitive of the 27 comparative assays showed a delay in HBsAg detection by roughly 2 weeks.
Obtained analytical sensitivities are summarized in the following table. Compared to Enzygnost® HBsAg 5.0 and to further 16 CE-marked HBsAg assays Enzygnost® HBsAg 6.0 is the device with the highest analytical sensitivity. Results from the future WHO HBV genotype panel confirm the high sensitivity for HBsAg of genotypes A-H.
The specificity of Enzygnost® HBsAg 6.0 was 99.92% and 99.87% for serum and plasma samples, respectively. Improved robustness against potentially interfering samples could be derived.

Table 1: Obtained analytical sensitivities

Standard or sensitivity panel	Subtype	Enzygnost® HBsAg 6.0	Enzygnost® HBsAg 5.0	Most sensitive competitive assay
PEI 1,000 U/mL (PEI-U/ml)	ad	0.007	0.007	0.009
PEI 50,000 U/mL (PEI-U/ml)	ay	0.012	0.010	0.018
"Ag HBs" SFTS 2005 (ng/ml)	adw2+ayw3	0.039	0.064	NA[a]
WHO 00/588 (IU/ml)	adw2	0.011	0.014	0.030
PHA808 (IU/ml)	ad	0.007	0.009	NA[a]
	ay	0.006	0.008	NA[a]
Future WHO HBV genotype A-H panel (IU/ml)	adw2, adw4, adr, ayw2, ayw3, ayw4	0.012 ± 0.003	NA[a]	NA[a]

[a] NA: not assigned

Summary/conclusion: Compared to a wide range of CE-marked assays, Enzygnost® HBsAg 6.0 is the assay with the highest sensitivity for detection of early HBV infection and of HBsAg in standard reference materials. Combined with the excellent specificity Enzygnost® HBsAg 6.0 is an optimal choice for routine HBsAg screening and diagnostic applications.

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