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The Lumipulse G HBsAg-Quant assay for screening and quantification of the hepatitis B surface antigen

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Qualitative HBsAg assay is used to screen HBV infection for decades. The utility of quantitative assay is also rejuvenated recently. We aimed to evaluate and compare the performance of a novel ultra-sensitive and quantitative assay, the Lumipulse assay, with the Architect and Elecsys assays.

As screening methods, specificity was compared using 2043 consecutive clinical routine samples. As quantitative assays, precision and accuracy were assessed. Sera from 112 treatment-naïve chronic hepatitis B patients, four patients undergoing antiviral therapy and one patient with acute infection were tested to compare the correlations. Samples with concurrent HBsAg/anti-HBs were also quantified.

The Lumipulse assay precisely quantified ultra-low level of HBsAg (0.004 IU/mL). It identified additional 0.98% (20/2043) clinical samples with trance amount of HBsAg. Three assays displayed excellent linear correlations irrespective of genotypes and S-gene mutations ($R^2 > 0.95$, $P < 0.0001$), while minor quantitative biases existed. The Lumipulse assay did not yield higher HBsAg concentrations in samples with concomitant anti-HBs.

Compared with other assays, the Lumipulse assay is sensitive and specific for detecting HBsAg. The interpretation of the extremely low-level results, however, is challenging. Quantitative HBsAg results by different assays are highly correlated, but they should be interpreted interchangeably only after conversion to eliminate the biases.

1. Background

Infection of hepatitis B virus (HBV) remains a severe threat to the public health worldwide, especially in the Asia-pacific region (Chen et al., 2000). For example, the prevalence of hepatitis B surface antigen (HBsAg) is 7.18% in China; accordingly there are ca. 93 million HBV carriers, among whom 30 million are patients with chronic hepatitis B (CHB) (Liang et al., 2009; Lu and Zhuang, 2009). CHB patients are at high risk of progressing to cirrhosis, liver failure and hepatocellular carcinoma (HCC) (Fattovich et al., 2008).

As a hallmark of HBV infection, serum qualitative HBsAg assay has been used for screening and diagnosis of HBV infection for decades. It still plays an important role even in the era of nucleic acid testing (NAT), especially for the resource-limited countries with a high prevalence of HBV infection (Allain and Cox, 2011). Recent studies also indicate the clinical significance of the quantitative HBsAg assay. For example, HBsAg levels vary in different phases of the natural history of HBV infection (Tseng and Kao, 2013). Baseline HBsAg < 1000 IU/mL and HBsAg annual decrease >0.3 log10 IU/mL were associated with HBsAg seroclearance. HBsAg ≥ 1000 IU/mL and HBV DNA ≥ 200 IU/mL could be combined to identify patients with risk of switching from inactive carrier to reactivation status (Martinot-Peignoux et al., 2013). The cumulative risk for cirrhosis and HCC rises with the increase of serum HBsAg levels (Lee et al., 2013). On the other hand, baseline or on-treatment HBsAg level can be potentially applied to predict the treatment outcome. Our previous study indicated that serum HBsAg < 1500 IU/mL at week 12 and <2890 IU/mL at week 24 had

Abbreviations: HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; anti-HBc, antibodies against hepatitis B core antigen; anti-HBs, antibodies against hepatitis B surface antigen; NAT, nucleic acid testing; CHB, chronic hepatitis B; HCC, hepatocellular carcinoma; OBI, occult HBV infection; PEG-IFN, peginterferon; NA, nucleos(t)ide analogue; ETV, entecavir; cccDNA, covalently closed circular DNA; CV, coefficient of variation.

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negative predictive values >90% for HBV e antigen (HBeAg) seroconversion at week 48 in patients treated with peginterferon (PEG-IFN) (Ma et al., 2010). The absence of HBsAg decline during PEG-IFN treatment helps identify the non-responders and determine the early stopping rule during PEG-IFN treatment (Rijckborst et al., 2012). Although the decline of HBsAg during the nucleos(t)ide analogue (NA) treatment seemed not so drastic (Zoulim et al., 2015), baseline HBsAg <1000 IU/mL and on-treatment HBsAg reduction >0.166 log IU/mL per year were optimal cutoff levels for predicting the long-term HBsAg loss (Seto et al., 2013).

Compared with HBV DNA testing, HBsAg assay is simple, cheap and provide results rapidly. There have been several commercial quantitative assays available (Burdino et al., 2014; Lee et al., 2012; Sonneveld et al., 2011). A novel HBsAg quantitative assay, the Lumipulse G HBsAg-Quant assay, has been recently developed (Matsubara et al., 2009). It is based on a new chemiluminescent enzyme immunoassay technology, claiming an ultra-sensitivity of 0.005 IU/mL. The assay is performed automatically on the Lumipulse G1200 immunoanalyzer (Choi et al., 2013). Trace amount of serum HBsAg could be detected even after the seroconversion (Shinkai et al., 2013). In this study, we evaluated and compared the performance of the Lumipulse G HBsAg-Quant with two commonly used assays, the Architect HBsAg and the Elecsys HBsAg II Quant assays in various clinical settings.

2. Materials and methods

The study was approved through the Peking University People's Hospital Ethical Committees.

2.1. HBsAg assays

The principle and procedure of the Lumipulse G HBsAg-Quant assay (Fujirebio, Tokyo, Japan) was described previously (Shinkai et al., 2013). Samples with results exceeding the upper limit was retested through 100-, 200- or 1000-fold dilution. HBsAg quantification using the Architect HBsAg assay (Abbott Ireland, Sligo, Ireland) and Elecsys HBsAg II Quant assay (Roche Diagnostics, Mannheim, Germany) was performed according to the manufacturer's instruction. The characteristics of the assays are summarized in Table 1.

2.2. HBsAg confirmatory tests

The presence of serum HBsAg was confirmed using the HBsAg confirmatory kits. HBsAg detected using the Lumipulse HBsAg assay was confirmed by the Lumipulse HBsAg confirmatory assay (Fujirebio, Tokyo, Japan), and the HBsAg detected using the Architect HBsAg assay was confirmed by the Architect HBsAg Confirmatory V.1 assay (Abbott, Wiesbaden, Germany). Polyclonal neutralizing antibodies against HBsAg were used to bind the immunodominant epitopes and thereby block the binding sites for the capture antibodies used in the HBsAg assays. Briefly, the sample with repeatedly positive HBsAg results was split as two aliquots. Reagent containing the neutralizing antibodies and the control reagent were added in parallel. HBsAg concentrations were measured after thorough mixing and incubation, and then the inhibition rate was calculated.

$$\text{Inhibition rate}\,(\%) = \frac{C_A - C_B}{C_A}.$$

where C is the HBsAg concentration (mIU/mL) for the Lumipulse assay, and sample/cutoff ratio (S/Co) for the Architect assay; A is the control reagent; B is the reagent containing neutralizing antibodies.

Table 1
Characteristics of the three HBsAg quantitative assays.

Assay	Analyzer	Principle	Technology (tracer)	Pre-treatment	Reaction sample volume	Assay duration	Linear range (analytical sensitivity)	On-board dilution	Traceability (NIBSC code)
Abbott Architect HBsAg	Architect $i2000_{SR}$	Sandwich principle, capture mAbs and polyclonal detection antibodies	CMIA (acridinium)	None	75 μL	29 min	0.05–250 IU/mL (0.05 IU/mL)	1:500 with recalcified negative human plasma	WHO first International standard, subtype ad (80/549)
Roche HBsAg II Quant	Molecular E170	Sandwich principle, two capture mAbs and a mixture of mAbs and polyclonal antibodies	ECLIA (ruthenium)	None	50 μL	18 min	0.05–130 IU/mL (0.05 IU/mL)	1:400 with buffered negative human serum	WHO second international standard, subtype adw2, genotype A (00/588)
Fujirebio Lumipulse G HBsAg-Quant	Lumipulse G1200	Sandwich principle, two capture mAbs and two detection mAbs	CLEIA (AMPPD)	Yes, to disrupt viral particles and dissociate HBsAg from HBsAg-anti-HBs complexes	100 μL	29 min	0.005–150 IU/mL (0.005 IU/mL)	1:100, 1:200 or 1:1000 with NaCl and Tris bufffer	WHO second international standard, subtype adw2, genotype (00/588)

CMIA, chemiluminescent microparticle immunoassay; ECLIA, electrochemiluminescence immunoassay; CLEIA, chemiluminescent enzyme immunoassay; mAb, monoclonal antibody; AMPPD, 3-(2′-spiroadamantane)-4-methoxy-4-(3″-phosphoryloxy)phenyl-1,2-dioxetane disodium salt