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SPECIALISED MEDICAL PATHOLOGY

# Evaluation of a new highly sensitive, qualitative and quantitative, Hepatitis B surface antigen assay.

Thoai Duong LY, Catherine COIGNARD (Laboratoire Biomnis, Ivry sur Seine, France)

## BACKGROUND AND OBJECTIVES

HBV surface antigen (HBsAg) is the established serological marker routinely used for the diagnosis of acute or chronic HBV infection and screening of blood or organ donors.

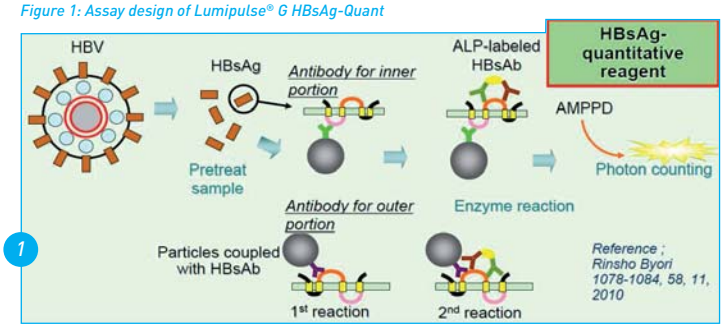
The European guidelines require a detection threshold below 130 mIU/mL for the CE marking of all HBsAg assays. Moreover, many CE marked HBsAg assays have a limit of detection near to 50 mIU/mL. In 2014, a new HBsAg assay (Lumipulse® G HBsAg-Quant, Fujirebio) was CE marked. The measurement range of this new assay is 5 – 150000 mIU/mL according to package insert.

We here aimed to investigate the sensitivity and the correlation of this new assay compared to that of the qualitative (Architect HBsAg Qualitative II, Abbott) and quantitative (Architect HBsAg-QT, Abbott) assays respectively.

## ASSAYS

Lumipulse® G HBsAg-Quant is an assay for the qualitative and quantitative detection of HBsAg based on CLEIA technology by a two-step sandwich immunoassay method (Fig 1)

*Figure 1: Assay design of Lumipulse® G HBsAg-Quant*

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*Lumipulse® G HBsAg-Quant*

*The characteristics of the 3 HBsAg assays are summarized in the table 1.*

| Manufacturer | Fujirebio | Abbott | |
|---|---|---|---|
| Platform | Lumipulse® G 1200 | Architect | |
| Name | HBsAg-Quant | HBsAg Qualitative II | HBsAg-QT |
| Assay | Qualitative and Qualitative | Qualitative | Quantitative |
| CE marked | 2014 | 2011 | 2003 |
| Solid phase Ab | 2 MAb | 2 MAb | 2 MAb |
| Conjugate Ab | 2 MAb | PAb | PAb |
| Sensitivity mIU/ml[a] | ≥ 5 | ≥ 30 | ≥ 50 |

*MAb: monoclonal antibody PAb: polyclonal antibody*
*[a] according to package insert*

## SAMPLES

In total, 268 samples were tested consisting of the following panels.

- Panel 1: WHO international standard HBsAg NIBSC (03/262) in 9 dilutions from 0 to 1,650 mIU/ml (n=9);
- Panel 2: 1st WHO Genotypes PEI panel 6100/09 (n=15)
- Panel 3: 4 seroconversion panels: PHM 911, 915, 916 and 935A (n=69)
- Panel 4: 82 samples of genotype A to H: 21A, 8B, 18C, 17D, 6E, 10F, 1G and 1H (n=82)
- Panel 5: 93 unselected samples submitted to the laboratory for the quantification (n=93).

## RESULTS

Using the WHO HBsAg NIBSC standard, the limit detection of Architect HBsAg qualitative II and Lumipulse® G HBsAg-Quant was determined to be 20 and 4.6 mIU/ml, respectively. Lumipulse® was able to detect the first positive sample in all 4 seroconversion panels (one or two bleeds before Architect HBsAg qualitative II).

All 97 genotype samples (panels 2 and 4) were detected by the 3 assays. Four and three samples in the panel 5 (n=93) were not quantified by Architect QT and Lumipulse® assays, respectively.

*The following table 2 summarizes the results.*

| | Architect Qualitative HBsAg II | Lumipulse® HBsAg-Quant | Architect Quantitative HBsAg |
|---|---|---|---|
| *Limit of detection* | | | |
| WHO Int standard (mIU/ml) | 20 | 4.6 | 50 (claimed) |
| *Seroconversion panels* (n=69) | *Number positive samples (Bleed day with the first positive result)* | | |
| PHM 911 (n= 25) | 6 (77) | 8 (70) | |
| PHM 915 (n=13) | 10 (14) | 12 (7) | |
| PHM 916 (n=11) | 3 (62) | 4 (55) | |
| PHM 935A (n=20) | 14 (21) | 16 (14) | |
| Total positive samples | 33 | 40 | |
| *Genotypes* (n=97) | | *Number positive samples* | |
| PEI panel (n=15) | | 15 | 15 |
| Panel 4 (n=82) | | 82 | 82 |
| *Quantitative routine samples* Panel 5 (n=93) | | 90 | 89 |
| *Statistical analysis* ($log_{10}$ mIU/ml) | | n = 186 | |
| Correlation | | r = 0.994 | |
| Bland-Altman | | - 0.0077 95% CI: - 0.0374 to 0.0221 | |

With 186 quantified samples, Lumipulse® G HBsAg-Quant and Architect-QT with measuring interval in $log_{10}$ mIU/ml of 1.64 to 8.08 and 1.84 to 7.51 respectively correlated by r = 0.994; Bland–Altman analysis agreement of mean difference was -0.0077 $log_{10}$ mIU/mL (95% CI: - 0.0374 to 0.0221).

In 88.7% of paired samples the difference between the two assays was ≤0.25 $log_{10}$ mIU/ml, while in 94.6% ≤0.30 $log_{10}$ mIU/ml and in 96.2% ≤0.40 $log_{10}$ mIU/ml (Fig 2).

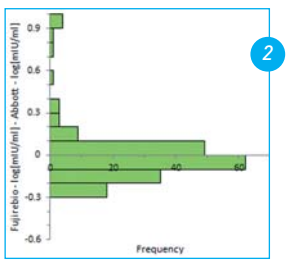


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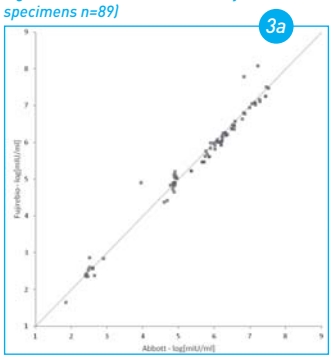
*Figure 2: Frequency histogram of overall observed differences between Lumipulse® and Architect measurements (in $log_{10}$ mIU/mL, n=186)*

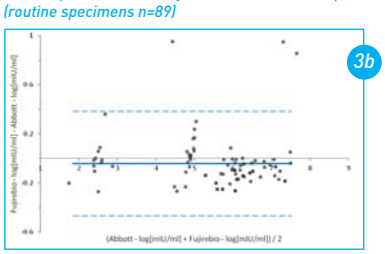
On unselected routine samples, the agreement between the two quantitative assays (Fig 3a and Fig 3b n=89 quantified by both) was high too (correlation r=0.989) and the mean difference was estimated to be -0.042 (standard deviation 0.218).

*Figure 3a: Correlation between Fujirebio and Abbott quantitative assays on unselected samples (routine specimens n=89)*



3a

*Figure 3b: Difference plot between Fujirebio and Abbott quantitative assays on unselected samples (routine specimens n=89)*



3b

We investigated the potential impact of HBV genotypes on measurement differences for quantifying HBsAg using panels 2 (PEI n=15) and 4 (n=82). The difference means with standard error bar are represented in Fig 4 (95 % interval confidence in diamond). Pair-wise comparisons were performed with Tukey adjustement for multiple comparisons of means. Only genotype A and genotype C demonstrated significantly difference means (p= 0.02 at 5% significance level). Note the within-genotype C variability is high.

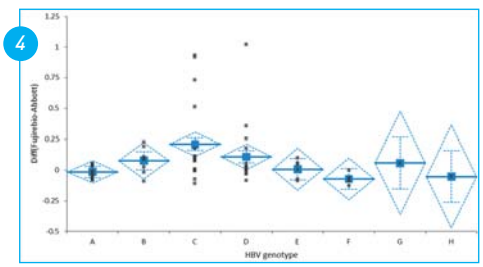



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*Figure 4: Difference means by HBV genotypes between Fujirebio and Abbott quantitative assays (n=97)*

## CONCLUSION

This study shows that the Lumipulse® G HBsAg-Quant assay revealed a higher sensitivity than the Architect HBsAg qualitative II. There is a high correlation and agreement between quantitative Lumipulse® G HBsAg measurements and Architect-QT quantitative assay. This new assay is suitable for routine clinical use and can be applied for HBsAg quantification in clinical practice.

Poster. 18th Annual Meeting of the European Society for Clinical Virology, 9 – 12 September 2015 Edinburgh