# Performance of an Ultra-sensitive HBsAg Assay


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## Introduction

Hepatitis B surface antigen (HBsAg) is the envelope protein of hepatitis B virus (HBV). Its presence in blood for more than 6 months is indicative of chronic hepatitis B (CHB). HBsAg detection is used for diagnosis and screening of blood products, and quantification is used to predict functional cure, defined as HBsAg loss with or without seroconversion. Conventional HBsAg assays have a limit of detection (LOD) around 0.05 IU/mL and may miss some diagnoses such as occult HBV.

Aim: The purpose of this study is to evaluate the analytical and clinical performance of an ultrasensitive (US) qualitative HBsAg assay (ABBOTT ARCHITECT HBsAg Next qualitative assay).

## Methods

1) Simple Precision:
Simple precision testing was achieved by evaluating 5 pooled specimen panels and controls. Each panel member was tested in 20 replicates in one run on the ARCHITECT i1000SR using the Abbott's HBsAg Next Qualitative assay.

2) Sample Carryover:
This study was performed to demonstrate elimination of clinically significant sample carryover. A high-level reactive samples at a concentration 147,300 IU/mL was tested followed by three HBsAg negative samples. All the four samples were measured by HBsAg Next in singleton.

3) Assay Limit of Detection:
The aim of this section of the study was to verify the lower limit of detection (LLOD) of the assay.
Two known international standards, 07-286* and 12-226**, were diluted to 0.005 IU/mL using ARCHITECT HBsAg Next Confirmatory Manual Diluent. These were then tested in duplicate over 5 days. In addition, the 2 reference standards were serially diluted 1 in 2 from 0.005 to 0.00025 IU/mL and tested to determine whether 0.005 was the true LLOD.

* CE Marked Material Monitor Sample for HBsAg 0.05IU/ml NIBSC code: 07/286-xxx subtype ad, c* CE Marked Material Monitor Sample for HBsAg 0.05IU/ml NIBSC code: 07/286-xxx subtype ad, calibrated against the 1st International standard for HBsAg (NIBSC Code Number 80/549, containing 100IU/vial HBsAg)
** WHO Third International Standard for HBsAg (HBV genotype B4, HBsAg subtypes ayw1/adw2)

4) Patient Specimens:
124 samples from 101 patients were tested. These included samples from patients with: past infection and apparent loss of HBsAg; occult hepatitis B infection; treatment cessation (STOP study) and subsequent loss of HBsAg; and negative samples. Samples that were reactive were (when specimen volume allowed) repeated and then confirmed with neutralisation, unless they were known positive samples from prior testing.

5) Prospective Study:
39 samples, which were referred for quantitative HBsAg and <0.05 IU/mL in the comparator (Roche ElecSys) assay, were tested prospectively.


Acknowledgements:

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We would also like to acknowledge the STOP study (St Vincent's Hospital, Melb.) and other cohort investigators.


## Results

### 1) Simple Precision

Table 1: Results of 20 replicates of each sample category to confirm precision.

| Sample | Range (S/CO) | Average (S/CO) | Standard deviation (S/CO) |
|---|---|---|---|
| Abbott negative panel | 0.320 – 0.412 | 0.365 | 0.027 |
| VIDRL patient negative | 0.294 – 0.976 | 0.400 | 0.144 |
| Abbott positive panel | 2.860 – 4.021 | 3.101 | 0.235 |
| VIDRL Low positive Patient | 1.000 – 1.209 | 1.093 | 0.0677 |
| VIDRL High positive Patient | 14.360 – 18.730 | 15.697 | 1.013 |

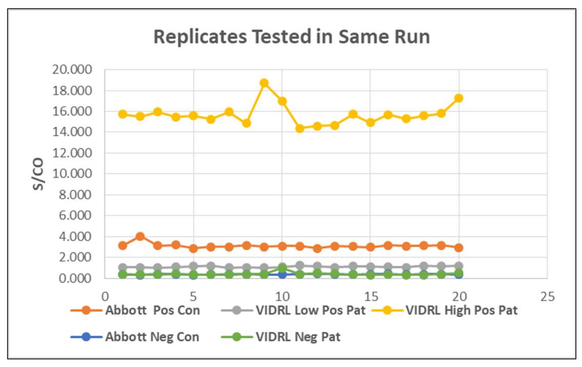


Figure 1: Replicates demonstrating simple precision

**2) Sample Carryover** was initially detected but resolved by implementation of Milton for decontamination of the instrument.

**3) Assay Limit of Detection** was confirmed as 0.005 IU/mL. Further dilutions below 0.005 IU/mL did not yield reactive results.

Table 2: Results, expressed as a ratio of signal/cut-off (S/CO) of the 2 international standards

| Standard | S/CO Range | S/CO Average | S/CO Standard Deviation |
|---|---|---|---|
| 07-286 | 1.4 – 1.63 | 1.51 | 0.07 |
| 12-226 | 1.25 – 1.59 | 1.40 | 0.11 |

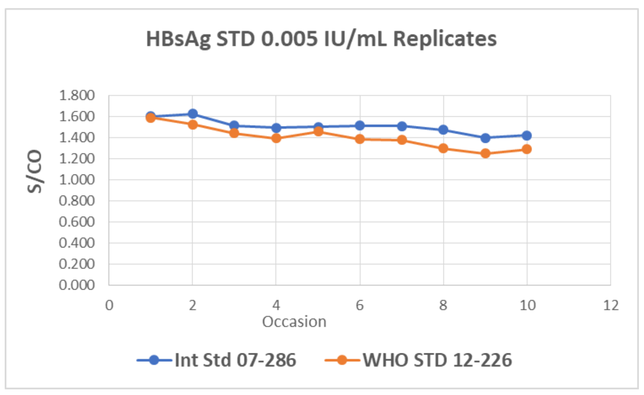


Figure 2: Results of replicates of the International standards diluted to 0.005 IU/mL.

## Prospective Study Results

Of 39 HBsAg-negative samples in the ROCHE ElecSys comparator assay and tested prospectively, 14 (36%) were reactive in the US Next assay.
10/11 of these were confirmed as positive by neutralisation.

## Discussion

The Abbott US Next assay was simple to perform, highly sensitive and reproducible (Figure 1, Table 1). The assay was able to qualitatively detect the 2 HBsAg international standards diluted to 0.005 IU/mL on each of the 20 replicates (Figure 2).
19 samples from patients with past history of CHB (including Pacific Island patients) were detected in the US Next assay and not detected by conventional assays(Table 3). In addition, 1 of 4 occult samples was detected in the US Next assay, although an additional sample from the same patient collected 7 months later, was undetectable. Another sample from a patient with occult HBV was non-reactive, despite having a detectable viral load of 25 IU/mL.
It was interesting that the assay was able to detect HBsAg in 1 of the 6 patients that had ceased nucleoside analogue treatment and subsequently lost HBsAg (determined by a conventional HBsAg assay), confirming the highly sensitive nature of the assay. However, the result did not alter the outcome (merely delayed it) for this patient with subsequent samples yielding non-reactive results.
Of 9 patient samples from HIV-HBV coinfection who demonstrated HBsAg loss, 1 was reactive and confirmed positive. The US Next assay was also able to prospectively detect HBsAg in 36% samples that were undetected by the comparator assay.

## Conclusion

The qualitative US Next assay was simple, reproducible and highly sensitive compared to comparator assays. Overall 38 samples that tested negative with conventional assays, were reactive, in the US Next assay, although not all samples were confirmed positive by neutralisation.

While the results shown in this study endorse the sensitivity of the US Next assay, further research is warranted to explore the clinical relevance of detection of low HBsAg levels in known CHB patients.

Nevertheless implementation of this assay may prove useful for diagnosis of occult HB infection, early acute HBV infection and for transfusion screening. Furthermore it is anticipated that a quantitative US assay would be advantageous in predicting HBsAg loss and improving laboratory workflow.

## Patient Samples

| Category | DIASORIN LIAISON IU/mL | ROCHE ELECSYS COBAS e411 IU/mL | No. samples tested | Non-reactive samples | Reactive samples | Confirmed by neutralisation | Not confirmed by neutralisation |
|---|---|---|---|---|---|---|---|
| Pacific Island samples - negative* | <0.03 - 0.05 | <0.05 - <0.1 | 38 | 34 | 4 | 1 | 2 |
| Occult samples | | <0.05 | 4 | 3 | 1 | 1 | |
| STOP Study# samples (HBsAg loss) - negative | | <0.05 - <1 | 15 | 13 | 2 | 1 | 1 |
| Neg samples with history CHB | | <0.05 - <1 | 34 | 19 | 15 | 5 | 3 |
| Neg samples with no history CHB## | <0.03 | <0.05 | 24 | 23 | 1 | | 1 |
| HIV-HBV coinfected patients with HBsAg loss | | <0.05 | 9 | 8 | 1 | 1 | |
| **TOTAL** | | | **124** | **100** | **24 (19%)** | **9 (56%)** | **7** |

Table 3: US Next results of patient samples that tested negative in the comparator assay