Performance Evaluation of the ADVIA Centaur Quantitative HBsAg Assay*

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Abstract
Background: Hepatitis B virus (HBV) is endemic throughout the world and is the major cause of liver disease. Hepatitis B surface antigen (HBsAg) is a distinctive serological marker of acute or chronic hepatitis B infection. Serum HBsAg levels are indirectly correlated with the control of the infection: the higher the infection control, the lower the serum HBsAg level. We report the analytical performance of a new quantitative assay for HBsAg (QHBs) on the ADVIA Centaur® Immunoassay System.

Methods: The LoB, LoD, and LoQ of the ADVIA Centaur QHBs assay were evaluated by testing 5 HBsAg-negative and 10 low-positive samples per CLSI document EP17-A2. Accuracy was assessed versus the WHO 3rd International Standard (12/226). High-dose hook effect was evaluated up to a concentration of 3.5 mg/mL of HBsAg. Linearity was evaluated per CLSI document EP6-A. Precision was determined using a 20-day protocol employing two systems and two runs per day. HBsAg results are reported in IU/mL, with an assay range for undiluted samples of the LoQ to 125 IU/mL.

Results: The ADVIA Centaur QHBs assay was determined to have an LoB of 0.006 IU/mL, an LoD of 0.012 IU/mL, and an LoQ of 0.020 IU/mL. No hook effect was observed up to a concentration of 3.5 IU/mL. The assay was linear across the reportable range of 0.020 IU/mL to 125 IU/mL. Extended range was achieved through the use of automated onboard dilutions of 1:500 and 1:2500, with 84% of HBsAg-positive samples reading inside the assay range at a 1:500 dilution. The QHBs assay had a total SCV of <5% across the extended assay range over a 20-day period with three lots of reagents.

Conclusions: The results of these studies show state-of-the-art performance for the fully automated ADVIA Centaur QHBs assay.

Background
Hepatitis B virus (HBV) is endemic throughout the world and is a major cause of liver disease. Hepatitis B surface antigen (HBsAg) is a distinctive serological marker of acute or chronic hepatitis B infection. Serum HBsAg levels are indirectly correlated with the control of the infection: the higher the infection control, the lower the serum HBsAg level. We report the analytical performance of a new quantitative assay for HBsAg (QHBs) on the ADVIA Centaur® Immunoassay System.

Methods
Principal of the procedure

The ADVIA Centaur QHBs assay is a multicapture sandwich assay using magnetic latex particles and a proprietary acridinium ester (AE) for chemiluminescent detection. Sample is initially diluted 1:500 with an onboard diluter. A portion of diluted sample (100 µL) is incubated with capture and detection monoclonal antibody reagent to form an immunocomplex. An additional detection monoclonal antibody is added to the sample followed by the magnetic particles to bind the HBsAg-antibody immunocomplexes. The solid phase particles are washed and treated with acid and base reagents to inactivate chemiluminescence. The magnitude of the relative light units (RLU) is proportional to the sample HBsAg concentration.

LoD, LoB, LoQ
• CLSI protocol EP17-A2
  • LoB: Determined nonparametrically by rank order, three reagent lots, n = 120.
  • LoD: Determined nonparametrically. Five low (0.030 IU/mL, target based on the WHO 2nd IS) HBsAg samples, n = 75 total replicates per sample/lot, 5 days, three reagent lots.
  • LoQ: Determined nonparametrically at a TAE of 35%. Five low HBsAg samples targeted to 0.050 IU/mL (based on the WHO 2nd IS), n = 70 total replicates per sample/lot, 10 days, three reagent lots.

Accuracy
• WHO 3rd International Standard for HBsAg (NIHSC code 12/226)
• Two-fold serial dilution of WHO Standard prepared at 47.428 IU/mL
• Observed dose recovery of singleton determination for each dilution

High-dose hook
• HBsAg AD and AY subtypes at 3.5 mg/mL
• Samples run at 1:500 dilution per algorithm

Precision
• CLSI protocol EP-5-A3
• Three reagent lots
• Two ADVIA Centaur systems, 20 days, n = 80 replicates per sample

Linearity
• CLSI EP17-A2
• Added mixture of two samples to create nine samples total
• Three reagent lots, three replicates per sample

Results
LoD, LoB, LoQ

Table 1. Observed lower limit of reportable results for the ADVIA Centaur QHBs assay.

<table>
<thead>
<tr>
<th>Reagent Lot</th>
<th>AD 0.002 (IU/mL)</th>
<th>AD 0.006 (IU/mL)</th>
<th>AD 0.012 (IU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot 1</td>
<td>≥0.006</td>
<td>≥0.012</td>
<td>+</td>
</tr>
<tr>
<td>Lot 2</td>
<td>≥0.006</td>
<td>≥0.012</td>
<td>+</td>
</tr>
<tr>
<td>Lot 3</td>
<td>≥0.006</td>
<td>≥0.012</td>
<td>+</td>
</tr>
</tbody>
</table>

Conclusion:
• Acceptable precision was demonstrated across multiple ADVIA Centaur Immunoassay Systems and reagent lots.
• Linearity was demonstrated across the full range of the assay.
• LoD values of 0.010, 0.009, and 0.012 IU/mL were obtained for each of three reagent lots.
• The assay LoQ was determined to be 0.019 IU/mL against the assay's primary calibration with a previous reagent lot.
• No high-dose hook was observed for antigen concentrations up to 3.5 mg/mL.
• 94% of samples fall within the calibration range of the assay.

Expected Values
Table 2. Purified HBsAg from AD and AY subtypes was prepared at 3.5 mg/mL (approx. 7 x 106 IU/mL) in negative human plasma basepool. These samples were run with three reagent lots of the ADVIA Centaur QHBs assay following the standard assay protocol. In all cases, the diluted results were greater than the calibration range of the assay (10.020-125.0 IU/mL).

Table 3. Results of a 20-day precision study following CLSI EP5-A3 using three reagent lots across two ADVIA Centaur systems showed repeatability precision of ±7% for samples run neat and ±7.3% for samples diluted on the system.

Conclusion:
• The precision and accuracy of the ADVIA Centaur QHBs assay support the accurate quantification of HBsAg in human serum and plasma.

Note: Not available for sale in U.S.

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Figure 1. The ADVIA Centaur Quantitative HBsAg assay format.