Evaluation of the Abbott HBV RUO Sequencing Assay Combined with Laboratory-Modified Interpretive Software

Jeffrey J. Germer, a Priya Abraham, b Jayawant N. Mandrekar, c Joseph D. C. Yao a

Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA a; Department of Clinical Virology, Christian Medical College, Vellore, India b; Department of Health Sciences Research, Mayo Clinic, Rochester, Minnesota, USA c

The Abbott HBV RUO Sequencing assay (Abbott Molecular Inc., Des Plaines, IL), which combines automated sample processing, real-time PCR, and bidirectional DNA sequencing, was evaluated for detection of nucleos(t)ide analogue (NA) resistance-associated mutations located in the hepatitis B virus (HBV) polymerase (Pol) gene. Interpretive software from the assay manufacturer was modified to allow interrogation of the overlapping HBV surface (S) gene sequence for HBV genotype determination and detection of immune escape mutations. Analytical sensitivity (detection and sequencing) of the assay was determined to be 103.9 IU/ml (95% confidence interval [CI], 80.0 to 173.3) for HBV genotype A. Testing of commercially available HBV genotype panels consisting of 23 individual members yielded complete agreement between expected results and results obtained from the laboratory-developed HBV genotype library. Excellent specificity was observed among clinical specimens with serologic or molecular markers for various unrelated blood-borne viruses (n = 6) and sera obtained from healthy, HBV-negative blood donors (n = 20). Retrospectively selected clinical specimens tested by a commercial reference laboratory HBV sequencing assay (n = 54) or the Trugene HBV Genotyping kit (n = 7) and the Abbott HBV RUO Sequencing assay showed minor differences in detection and reporting of NA resistance-associated mutations in 7 of 61 (11.5%) specimens but complete agreement of genotype results. The Abbott HBV RUO Sequencing assay provided a convenient and efficient assay workflow suitable for routine clinical laboratory use, with the flexibility to be modified for customized detection of NA resistance-associated mutations, HBV genotype determination, and detection of immune escape mutations from a single contiguous HBV sequence.

Globally, over 400 million individuals are chronically infected with hepatitis B virus (HBV) (1, 2). Ongoing HBV replication in these chronic carriers has been shown to increase their risk for disease progression, including the development of cirrhosis and hepatocellular carcinoma (3, 4), while infection with HBV genotype C has also been independently associated with a potential for more-severe liver disease (5–7). Treatment options for chronic hepatitis B have continued to expand and currently include the use of standard or pegylated interferon or nucleos(t)ide analogues (NA). However, recent reports of reduced effectiveness of interferon-based therapies in patients infected with HBV genotypes C or D compared to genotypes A or B (8–10), along with the well-documented potential for NA resistance, can complicate treatment decisions.

Among orthotopic liver transplant (OLT) recipients treated for end-stage liver disease due to chronic hepatitis B or at risk for de novo HBV infection, hepatitis B immunoglobulin (HBIG) has been used together with long-term NA therapy to prevent HBV reinfection of the graft (11, 12). Unfortunately, in addition to the potential for development of NA resistance with long-term use, extended use of HBIG in these transplant recipients has also been associated with the emergence of HBV immune escape mutants, resulting in reduced effectiveness of HBIG (13–16). Routine testing of HBV for resistance to NA (i.e., drug-resistant mutants) and HBIG (i.e., immune escape mutants) is likely to play an increasingly important role (along with viral load testing) in the management of these transplant recipients as well as patients chronically infected with HBV.

HBV genotype determination and detection of drug resistance and immune escape mutations by various laboratory-developed assays have been described extensively in the literature. Examples of some of the methods used in these laboratory-developed assays include direct sequencing, pyrosequencing, allele-specific real-time PCR, PCR combined with matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF), Invader technology, and microchip-based sequencing (17–22). Several research-use-only assays based on either direct sequencing (such as the Trugene HBV Genotyping kit; Siemens Healthcare Diagnostics Inc., Tarrytown, NY) or reverse hybridization (such as the INNO-LiPA HBV Multi-DR and INNO-LiPA HBV Genotyping kits; Innogenetics NV, Ghent, Belgium) are also commercially available. Each of these various assay formats has its own unique advantages and disadvantages that must be considered when selecting an assay for routine clinical laboratory use. For example, while sequencing-based assays generally offer greater flexibility in detection and reporting of new or unexpected mutations, hybridization-based assays may provide enhanced capability to detect mutant HBV subpopulations.

The Abbott HBV RUO Sequencing assay (Abbott HBV Seq) (Abbott Molecular Inc., Des Plaines, IL) is a relatively new assay developed specifically for the detection of NA resistance-associated mutations in HBV through bidirectional DNA sequencing of a portion of the HBV polymerase (Pol) gene’s reverse transcriptase

Received 10 August 2012 Returned for modification 5 September 2012 Accepted 13 October 2012 Published ahead of print 24 October 2012
Address correspondence to Joseph D. C. Yao, jdcyao@mayo.edu.
Supplemental material for this article may be found at http://dx.doi.org/10.1128/JCM.02155-12.
Copyright © 2013, American Society for Microbiology. All Rights Reserved.
doi:10.1128/JCM.02155-12
region (RT), and it is currently available for research use only in the United States. User modification of the HBV sequence interpretation criteria used in conjunction with Abbott HBV Seq can also allow the assay to be used to interrogate the overlapping HBV surface (S) gene sequence to determine the HBV genotype and identify immune escape mutations. The current study evaluated the performance characteristics of Abbott HBV Seq performed in conjunction with HBV sequence interpretation software modifications allowing HBV genotype determination and detection of immune escape mutations in addition to the detection of NA resistance from a single assembled HBV sequence.

MATERIALS AND METHODS

Reference materials. HBV panels containing dilutions of HBV genotype A were prepared from a commercially available OptiQuant HBV DNA quantification panel (AcroMetrix Corp., Benicia, CA) using NAT dilution matrix (AcroMetrix Corp.) as a diluent. The final replicate HBV panels used in the evaluation consisted of individual panel members with HBV DNA concentrations of 10, 50, 100, 1,000, 10,000, 100,000, and 10,000,000 IU/ml. These panels were tested by Abbott HBV Seq in triplicate on 4 separate days, yielding a total of 12 results at each HBV DNA level.

Admixtures of mutant HBV (derived from a clinical specimen containing HBV with mutations r180M and r204V previously determined by a laboratory-developed sequencing assay; Quest Diagnostics Nichols Institute, San Juan Capistrano, CA) combined with HBV of wild-type origin at 1:3, 1:4, and 1:5 ratios were prepared to simulate the presence of a minor population of an HBV mutant (at a final concentration of 500 IU/ml) in a background of wild-type HBV. These admixtures were each tested by Abbott HBV Seq in triplicate over multiple assay runs and analyzed for the presence of these clinically important mutations.

Two commercially available HBV panels, Teragenix HBV Genotype Performance Panel PHD350 (SeraCare Life Sciences, Inc., Milford, MA) and HBV DNA Genotype Performance Panel PHD201(M) (SeraCare Life Sciences, Inc.), were analyzed by Abbott HBV Seq used in conjunction with an integrated, laboratory-developed HBV genotype library (see details below) designed specifically for use with HBV sequence data generated by Abbott HBV Seq. Select members of the Teragenix HBV DNA Genotype Performance Panel PHD350 were diluted with commercially available normal human serum (AcroMetrix Corp.) as a diluent. The final replicate HBV panels containing dilutions of HBV genotype A to H, each at a final concentration of 500 IU/ml, PCR amplification results and corresponding HBV sequences were evaluated to assess the ability of Abbott HBV Seq to produce valid, interpretable sequences across all HBV genotypes and at low HBV DNA levels. Genotype results generated by the laboratory-developed HBV genotype library were also compared to the expected HBV genotype results (per SeraCare product inserts) for agreement. All reference panel members and analytical preparations were stored at −70°C until the time of testing.

Clinical specimens. Evaluation of the specificity of Abbott HBV Seq included testing sera from 20 unique, healthy, HBV-negative blood donors and 6 clinical serum or plasma specimens, each containing one of the following viral serologic or molecular markers: hepatitis A virus IgM antibody, hepatitis E virus IgM antibody, hepatitis C virus RNA, HIV-1 RNA, cytomegalovirus DNA, and Epstein-Barr virus DNA. For comparison of assay methods (see details below), 54 HBV DNA-positive stored clinical serum specimens previously analyzed for HBV genotype and NA resistance-associated mutations between July 2009 and August 2010 along with 7 stored serum specimens obtained from OLT recipients infected with HBV and previously analyzed for HBV genotype, NA resistance-associated mutations, and immune escape mutations in December 2001 (23) were selected retrospectively. Due to insufficient remaining specimen volume (<0.8 ml), 17 of 54 leftover clinical specimens and all 7 of the specimens obtained from OLT recipients required dilution with normal human serum (all final HBV DNA levels were ~500 IU/ml or greater) prior to testing by Abbott HBV Seq. All clinical specimens were stored at −70°C until the time of testing by Abbott HBV Seq.

Abbott HBV RUO Sequencing assay. Sample processing followed by real-time PCR amplification and detection was performed on the integrated Abbott m2000sp/m2000rt instrument system (Abbott Molecular Inc.) according to the assay manufacturer’s recently updated instructions for use, using the m2000 0.5 ml HBV Sequencing Version 2.0 software application and HBV RUO Sequencing Application CD-ROM version 2.0 (Abbott Molecular Inc.). A minimum specimen volume of 0.8 ml (0.5 ml plus 0.3-ml overfill) was required for automated processing by the m2000sp with the 0.5-ml input volume protocol. Detection and quantification of the 1,023-bp HBV amplification product was performed with the m2000rt, and amplification products were diluted for optimal sequencing performance as indicated by the associated test result comment. Four individual sequencing reactions (two forward and two reverse) were performed for each sample to provide bidirectional sequences spanning the Pol RT and S gene regions of interest (Fig. 1). All post-PCR cleanup and cycle sequencing procedures were performed on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA). Cycle sequencing products were generated with the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) and analyzed by capillary electrophoresis with a 50-cm capillary array on an Applied Biosystems 3130xl Genetic Analyzer (AB 3130xl; Applied Biosystems) according to the assay manufacturer’s instructions and analyzed with SeqScape Software, version 2.7 (Applied Biosystems).

Laboratory-modified data analysis software. DNA sequence data generated on the AB 3130xl were analyzed using a laboratory-modified version of the “HBV_SEQv2” software program (Abbott Molecular Inc.), an assay manufacturer-defined project template developed in SeqScape Software. In addition to producing assembled bidirectional HBV DNA sequences, the project template enabled performance of the assay manufacturer’s sequence validity checks and assisted in the identification of NA resistance-associated mutations of potential clinical interest occurring at 14 different amino acid positions ranging from codons r80 to rt250 (see Table S1 in the supplemental material).

Without altering the assay manufacturer’s basic analysis protocol and analysis settings contained in the HBV_SEQv2 template, the reference data group (RDG) of the template was expanded to include the following: (i) a laboratory-developed HBV genotype library associated with the “Validity Layer” or region of interest (ROI) (codons r70 to rt260; Fig. 1) and containing 8 individual reference sequences, each at a length of 575 bp and corresponding to HBV genotypes A to H (Table 1); (ii) a laboratory-developed “Immune Escape” ROI (678 bp in length) defining the overlapping HBV S gene sequence to be interrogated; and (iii) customized “NT and AA Variant” lists containing HBV immune escape mutations of potential clinical relevance in addition to the NA resistance-associated

FIG 1 The Abbott HBV RUO Sequencing assay (Abbott HBV Seq) combines real-time PCR amplification and detection with DNA sequencing utilizing multiple forward (Seq A and Seq B) and reverse (Seq C and Seq D) sequencing reactions. Assembled sequences were analyzed by using defined “Layers” or “Regions of Interest” (Validity, Nucleos(t)ide analogue resistance, and Immune escape) contained in the modified sequence interpretation software. The overlapping nature of the HBV genome permitted the simultaneous detection of immune escape mutations and HBV genotype determination in addition to the detection of mutations associated with nucleos(t)ide analogue resistance.

Germer et al.
mutations specified by the assay manufacturer. The laboratory-defined “Immune Escape” ROI was designed to specifically identify the HBV S gene mutants sD144A/G/H, and sG145A/E/K/R (see Table S2 in the supplemental material). All HBV genotype assignments were based on the best match between the sequence under interrogation and the 8 individual reference sequences representing HBV genotypes A to H, each edited to the minimum HBV DNA sequence segment required by the assay manufacturer to assemble a valid sequence for further analysis. NA resistance-associated and immune escape mutations were automatically identified by the software using the criteria listed in the customized “AA Variant” list and confirmed by visual inspection of the corresponding electropherograms produced by the AB 3130xl.

Systematic, manual alteration of an imported HBV genotype A sequence (GenBank X02763) was performed with the modified SeqScape project template to confirm its ability to correctly identify and flag all unique nucleotide substitutions (including possible IUPAC nucleotide ambiguity codes) associated with NA resistance-associated mutations at 14 different amino acid positions (specified by the assay manufacturer) and possible immune escape mutations at 2 different amino acid positions (specified by the laboratory-developed “Immune Escape” ROI) defined in the customized “NT Variant” and “AA Variant” lists (see Tables S1 and S2 in the supplemental material). Performance of the HBV genotype library was also assessed by importing and analyzing 3 well-characterized reference sequences of each HBV genotype (A to H) with the modified project template (see Table S3 in the supplemental material).

Comparison of assay methods. The 54 leftover clinical serum specimens selected for testing by Abbott HBV Seq were previously analyzed for HBV genotype and the presence of NA resistance-associated mutations by a laboratory-developed sequencing assay (Quest Diagnostics Nichols Institute). This laboratory-developed assay was based on nucleic acid extraction with the MagNA Pure LC (Roche Applied Science, Indianapolis, IN) followed by PCR amplification of a portion of the HBV Pol gene (25) with subsequent cycle sequencing reactions performed with the BigDye Terminator v.3.1 Cycle Sequencing kit (Applied Biosystems). HBV sequences were generated by capillary electrophoresis (AB 3130xl) and analyzed with Seqencher Software (Gene Codes Corp., Ann Arbor, MI) (R. M. Kagan, personal communication). The 7 stored clinical serum specimens obtained from OLT recipients and selected for testing by Abbott HBV Seq were previously analyzed with the Trugene HBV Genotyping kit (Siemens Healthcare Diagnostics, Tarrytown, NY) performed according to the assay manufacturer’s instructions (23).

Data analysis. Probit analysis (with a 95% hit rate) was used to estimate the analytical sensitivity or limit of detection (LoD) for Abbott HBV Seq (26).

RESULTS
Abbott HBV Seq assay performance. Analytical sensitivity and reproducibility of Abbott HBV Seq at various HBV DNA levels are shown in Table 2. Based on the number of replicate aliquots yielding a detectable and interpretable HBV sequence at each HBV DNA level, the LoD of Abbott HBV Seq was determined to be 103.9 IU/ml (95% confidence interval [CI], 80.0 to 173.3 IU/ml) by Probit analysis using a 95% hit rate. Despite this level of sensitivity, Abbott HBV Seq failed to reliably detect NA resistance-associated mutations (rt180M and rt204V at a final HBV DNA concentration of 500 IU/ml) when present at 1:3, 1:4, and 1:5 ratios along with wild-type HBV in admixture experiments. Specifically, these mutations were detected by Abbott HBV Seq in only 1 of 3 replicates at a mutant-to-wild-type ratio of 1:3. No mutation was identified in replicates at mutant-to-wild-type ratios of 1:4 and 1:5 by either automated or manual inspection of the individual electropherograms.

Specificity and cross-reactivity studies performed with serum specimens obtained from healthy blood donors (n = 20) along with clinical serum specimens containing markers for unrelated bloodborne viruses (n = 6) yielded HBV-negative PCR results by Abbott HBV Seq as expected.

Sequence analysis software performance. Manipulations of the nucleotide sequence of HBV genotype A (GenBank X02763) followed by analysis with the modified SeqScape project template verified the ability of the software template to correctly identify and flag all unique nucleotide substitutions (including possible IUPAC nucleotide ambiguity codes) associated with the customized “NT Variant” and “AA Variant” lists (see Tables S1 and S2 in the supplemental material). Analysis of 24 well-characterized and imported reference sequences representing HBV genotypes A to H yielded a single discordant result: an HBV reference sequence previously assigned to genotype D was misidentified as genotype A compared to the 573-bp HBV sequence segments contained in the laboratory-developed HBV genotype library (see Table S3 in the supplemental material). The previous genotype D assignment was confirmed by additional phylogenetic analyses (data not shown) performed with the entire HBV sequence (GenBank accession no. X68292). Subsequent analysis of 10 additional well-characterized sequences of HBV genotype D yielded the expected results without further genotype discordance (see Table S4 in the supplemental material).

Two commercially available HBV genotype panels (PHD350 and PHD201[M]) were tested by Abbott HBV Seq and analyzed with the modified SeqScape project template to further assess the performance of the assay and laboratory-developed HBV geno-

---

**Table 1** HBV reference sequences included in a laboratory-developed HBV genotype library designed for use with the Abbott HBV RUO Sequencing assay (Abbott HBV Seq) software.

<table>
<thead>
<tr>
<th>GenBank accession no.</th>
<th>HBV genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>X02763</td>
<td>A</td>
</tr>
<tr>
<td>D00330</td>
<td>B</td>
</tr>
<tr>
<td>AB033556</td>
<td>C</td>
</tr>
<tr>
<td>X02496</td>
<td>D</td>
</tr>
<tr>
<td>X75657</td>
<td>E</td>
</tr>
<tr>
<td>X75663</td>
<td>F</td>
</tr>
<tr>
<td>AF160501</td>
<td>G</td>
</tr>
<tr>
<td>AY909460</td>
<td>H</td>
</tr>
</tbody>
</table>

*a* All individual HBV sequences were edited to a length of 573 bp corresponding to codons rt70 to rt260, the minimum requirement for a valid, assembled sequence generated by Abbott HBV Seq. All data are from Yuen et al., 2007 (24).

---

**Table 2** Analytical sensitivity of the Abbott HBV RUO Sequencing assay (Abbott HBV Seq).

<table>
<thead>
<tr>
<th>Amt of HBV DNA (IU/ml)</th>
<th>No. of replicates tested</th>
<th>Replicates yielding a detectable and interpretable HBV sequence consistent with HBV genotype A*</th>
</tr>
</thead>
<tbody>
<tr>
<td>10,000</td>
<td>12</td>
<td>12 100 (73.5–100)</td>
</tr>
<tr>
<td>1,000</td>
<td>12</td>
<td>12 100 (73.5–100)</td>
</tr>
<tr>
<td>100</td>
<td>11b</td>
<td>10 90.9 (58.7–99.8)</td>
</tr>
<tr>
<td>50</td>
<td>12</td>
<td>7 58.3 (27.7–84.8)</td>
</tr>
<tr>
<td>10</td>
<td>12</td>
<td>1 8.3 (0.2–38.5)</td>
</tr>
</tbody>
</table>

*a* Probit value (95% hit rate) = 103.9 IU/ml (95% CI, 80.0 to 173.3).

*b* A single replicate result was excluded from this analysis due to a sample processing error.

---

January 2013 Volume 51 Number 1 jcm.asm.org
TABLE 3 Performance of the Abbott HBV RUO Sequencing assay (Abbott HBV Seq) among clinical specimens previously tested for HBV nucleos(t)ide analogue resistance by a laboratory-developed assay

<table>
<thead>
<tr>
<th>No. of specimens</th>
<th>Laboratory-developed assay results</th>
<th>Abbott HBV Seq results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rt80V, rt180 M, rt204I</td>
<td>rt80V, rt204I</td>
</tr>
<tr>
<td>1</td>
<td>rt173L, rt204I, rt207I</td>
<td>rt173L, rt204I</td>
</tr>
<tr>
<td>1</td>
<td>rt173L, rt180 M, rt204I, rt207I</td>
<td>rt173L, rt204I</td>
</tr>
<tr>
<td>2</td>
<td>rt180 M, rt204I</td>
<td>rt180 M, rt204I</td>
</tr>
<tr>
<td>1</td>
<td>rt180 M, rt184L, rt204V</td>
<td>rt180 M, rt184L, rt204V</td>
</tr>
<tr>
<td>1</td>
<td>rt204I</td>
<td>rt204I</td>
</tr>
<tr>
<td>1</td>
<td>None reported</td>
<td>rt214A, rt215S</td>
</tr>
<tr>
<td>1</td>
<td>None reported</td>
<td>rt215S</td>
</tr>
<tr>
<td>45</td>
<td>None reported</td>
<td>None reported</td>
</tr>
</tbody>
</table>

\(^a\) Laboratory-developed DNA sequencing assay performed at Quest Diagnostics Nichols Institute, San Juan Capistrano, CA.

\(^b\) Not detected by Abbott HBV Seq (diluted 1:2 prior to testing due to insufficient quantity).

\(^c\) Not included in Abbott HBV Seq database (possible association with famciclovir resistance).

\(^d\) Not included in laboratory-developed assay database (possible association with adefovir resistance).

differences in result reporting (4 specimens) and failure of Abbott HBV Seq to detect the rt180M mutation (2 specimens). While Abbott HBV Seq did yield valid, interpretable sequences from both of the last 2 specimens, both specimens required 1:2 dilution prior to testing due to insufficient specimen volume, which may have contributed to this discordance of assay results. An s145R immune escape mutation was also detected by Abbott HBV Seq in 1 of 54 (1.9%) clinical specimens, but no such mutation result was available from the reference assay for comparison.

There was excellent agreement between the results obtained by Abbott HBV Seq and those obtained by the Trugene HBV Genotyping kit among the 7 specimens obtained from HBV-infected OLT recipients (Table 4). The only notable discrepancy among the results was the failure of the Trugene HBV Genotyping kit to detect the NA resistance-associated mutations rt80V and rt80I in 2 of these 7 specimens. This discrepancy was the direct result of a limitation in assay design (coverage from codons rt99 to rt280 only) associated with the Trugene HBV Genotyping kit that has previously been noted by other investigators (19, 22).

DISCUSSION

With the development of new and more potent NA and interferon preparations for the treatment of chronic hepatitis B, there is a potential for increased clinical demand for HBV genotype determination and drug resistance testing. Current use of HBIG to prevent recurrent or de novo HBV infection in OLT recipients also suggests that the ability to detect HBV immune escape mutations would be useful to optimize management of OLT candidates and recipients who may be infected with HBV vaccine or HBIG escape mutants, respectively. To address these expanded clinical needs, we have developed a laboratory-modified SeqScape project template for use in conjunction with Abbott HBV Seq and evaluated the analytical and clinical performance of Abbott HBV Seq (using modified sequence interpretation) with both analytical standards and clinical specimens. In addition to highlighting and evaluating the expanded, user-defined capabilities of Abbott HBV Seq, this work is among the first published evaluations of this recently introduced and commercially available assay.

Use of a commercially available kit such as Abbott HBV Seq combined with automated sample processing (m2000sp), semiconductor real-time PCR (m2000rt), and capillary electrophoresis-based Sanger sequencing (AB 3130xl) provided a convenient and efficient assay workflow suitable for routine use in clinical testing. Abbott HBV Seq demonstrated excellent sensitivity (i.e.,

TABLE 4 Abbott HBV RUO Sequencing assay (Abbott HBV Seq) performance among clinical specimens previously tested by the Trugene HBV Genotyping kit (TG HBV)\(^a\)

<table>
<thead>
<tr>
<th>Specimen ID</th>
<th>TG HBV results</th>
<th>Abbott HBV Seq results</th>
</tr>
</thead>
<tbody>
<tr>
<td>OLT 1</td>
<td>C</td>
<td>rt180 M, rt204I</td>
</tr>
<tr>
<td>OLT 2</td>
<td>C</td>
<td>rt180 M, rt204V</td>
</tr>
<tr>
<td>OLT 3</td>
<td>C</td>
<td>rt204I</td>
</tr>
<tr>
<td>OLT 4</td>
<td>D</td>
<td>rt215S/P</td>
</tr>
<tr>
<td>OLT 5</td>
<td>C</td>
<td>ND</td>
</tr>
<tr>
<td>OLT 6</td>
<td>D</td>
<td>ND</td>
</tr>
<tr>
<td>OLT 7</td>
<td>A</td>
<td>ND</td>
</tr>
</tbody>
</table>

\(^a\) NA, nucleos(t)ide analogue; ND, none detected; ID, identification.

\(^b\) Located outside the HBV genomic region interrogated by TG HBV (codons rt99 to rt280).
104 IU/ml) among analytical standards prepared with HBV genotype A and provided additional evidence of comparable sensitivity and overall assay performance among strains representing HBV genotypes A to H, without the need for nested amplification. In addition, use of the SeqScape software template allowed the assay manufacturer’s sequence assembly process and sequence quality assessments to be performed automatically prior to sequence interrogation and subsequent identification of clinically relevant mutations using the customized “NT and AA Variant” lists and HBV genotype library. Finally, the incorporation of dUTP combined with the optional use of uracil-N-glycosylase (UNG) provides an additional level of carryover prevention that may be desirable for routine use of the assay in a clinical diagnostic laboratory.

Although the number of clinical serum specimens with clinically significant amino acid substitutions (RT and S mutations) was limited in this study, the ability of Abbott HBV Seq to successfully generate valid sequences suitable for analysis was independent of the number of such substitutions present. However, Abbott HBV Seq was subject to the inherent limitations of population-based Sanger sequencing chemistry. Specifically, the ability of the assay to detect minor populations was found to be limited, and confirmation of mutations by manual inspection of the individual electropherograms was generally complicated and time-consuming. In the current study, the populations in preparations containing HBV DNA levels of 1,500, 2,000, and 2,500 IU/ml with 33.3%, 25.0%, and 20% HBV mutant subpopulations (rt180M and rt204V), respectively, were not consistently detectable by the Abbott HBV Seq software or by manual inspection of individual electropherograms. However, our admixture experiments did present a significant challenge to the assay by testing various dilutions of wild-type HBV containing a subpopulation of a drug-resistant mutant present at a very low level (i.e., 500 IU/ml). Although population-based sequencing is capable of detecting mutant HBV subpopulations present at a minimum of 10% to 50% of the total population in a given clinical specimen (19, 27), one should be aware that detectability is dependent on both the relative abundance of the subpopulation and the total HBV viral load. A minor variant present at 30% of a total viral population of 1,500 IU/ml may not be detectable, while a variant present at 25% of a total population of 5,000 IU/ml may be detectable. It is also important to note that the potential advantages and clinical significance of an improved capability to detect minor populations of HBV drug-resistant mutants remain to be determined, given the uncertain influence of such minor viral populations on therapeutic response (17, 22, 28).

Compared to various sequence interrogation methods with improved detection capability for minor HBV variant populations (e.g., INNO-LiPA, allele-specific PCR, PCR combined with MALDI-TOF, and Invader), population-based sequencing methods still offer several important advantages. Unlike these alternative methods, sequencing-based methods are typically not affected adversely by unanticipated polymorphisms in the flanking regions surrounding the mutation sites of interest. These methods can also be updated or modified more easily to detect newly recognized mutations of interest than methods that are dependent on hybridization of sequence-specific primers or probes. This adaptability is a key feature of sequencing-based assays, since HBV sequence interrogation guidelines are not well standardized currently and may be subject to frequent changes and updates, while web-based HBV sequence interpretative tools cannot be validated easily for routine use in clinical testing.

Limited sequence variability among the 8 HBV genotypes (A to H) over the 573-bp segment (“Validity Layer”) of the HBV Pol/RT sequence resulted in an incorrect genotype assignment (genotype A) for an HBV reference sequence of genotype D. While this finding highlighted a potential limitation of our laboratory-developed HBV genotype library (see Table S3 in the supplemental material), it should be noted that genotype assignments based on the highest identity score (i.e., best match) are not intended to replace formal phylogenetic analysis of HBV sequences for investigational purposes. Additionally, the creation of our customized interpretive software was intended only to demonstrate the potential capabilities of such customized assay features. Establishment of a minimum identity score or the addition of more HBV sequences to the customized HBV genotype library may result in improved accuracy of HBV genotype assignments.

In summary, Abbott HBV Seq was found to be suitable for detection of HBV NA resistance-associated mutations in clinical specimens. Use of the laboratory-modified interpretive software in conjunction with Abbott HBV Seq not only permitted detection of a comprehensive list of HBV NA resistance-associated mutations but also allowed accurate HBV genotype determination and detection of immune escape mutations from the same contiguous HBV sequence.

ACKNOWLEDGMENTS

This work was supported in part (kits and consumables) by Abbott Molecular Inc. I.D.C.Y. has received research grants from Abbott Molecular Inc. and served as an advisory board member for Abbott Molecular Inc.

We are grateful to Ron M. Kagan for providing details of the laboratory-developed assay performed at Quest Diagnostics Inc.

REFERENCES


