EVALUATION OF THE EVIVAR SEQHEPB SYSTEM FOR DETERMINATION OF HEPATITIS B VIRUS GENOTYPE, RESISTANCE-ASSOCIATED MUTATION DETECTION, AND RESISTANCE INTERPRETATION

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ABSTRACT

Background: The Evivar SeqHepB system is an online HBV sequence analysis tool used to determine genotype, identify antiviral resistance-associated mutations, and predict resistance to lamivudine, telbivudine, entecavir, adefovir, and tenofovir. The SeqHepB system is linked to a phenotypic database of proven HBV resistance-associated mutations. The objective of this study was to compare the SeqHepB system to a laboratory-developed analysis method, which includes genotype determination and mutation detection using ARUP SeqHeap v2.0 software with resistance interpretations based on 2009 EASL guidelines.

METHODS:

- Consensus sequences generated from 86 patient plasma samples positive for HBV DNA according to the methods described in Mallory et al. were included in the study. Of these, 58 were considered routine and 28 were considered challenging to analyze due to unusual resistance mutation patterns, presence of numerous mixed positions, indels, etc. Sequences from an additional 15 samples representing rare genotypes E-H were also evaluated by both methods. To establish the capacity of the SeqHepB system for analyzing mixtures of variants, sequence data from mixtures of one genotype E sample and one genotype A sample at ratios of 60:40, 70:30, 80:20 and 90:10 were also evaluated.

- Results: Of the 86 sequences previously described, 85 and 86 were successfully analyzed using the SeqHepB system and the laboratory-developed method, respectively. One sequence with a large deletion was rejected by the SeqHepB system. Genotype calls by both methods were 100% concordant. However, the SeqHepB system assigns reduced sensitivity to entecavir (ETV) resistance mutations compared to the laboratory-developed method. Resistance discrepancies included assignment of reduced sensitivity to ETV by SeqHepB for the resistance pattern L180M + M204V/I + V173L while EASL designates full resistance and the designation of reduced sensitivity to tenofovir (TFV) for the resistance pattern L180M + S202G + M204I. Five of eight discrepancies were concordant by both methods for 58 of 58 routine sequences and 22 of 27 challenging sequences. One sequence with a large deletion was rejected by the SeqHepB system. Genotype calls by both methods were 100% concordant.

RESULTS:

- Table 1. Genotype Concordance for Challenging and Routine HBV RT Patient Sequences

Table 2. Comparison of Resistance Mutations Detected and Interpretations based on LDA and SeqHepB

Table 3. HBV Resistance Interpretation Algorithm for Laboratory-developed Analysis (LDA)

CONCLUSIONS:

- Genotype calls were concordant by both the LDA and Evivar SeqHepB methods for 100% of routine sequences and 87% successfully analyzed challenging sequences. Analyzing sequences with large indels or numerous mixed positions may confound either method.

- Resistance associated mutations were detected in 23 of 86 patient sequences. In three cases, a single mutation was detected by one method and no mutations were detected by the other method. Mutations included in the Evivar SeqHepB algorithm that are not included in the LDA/EASL algorithm are A181V and D230E. Other variations at these positions (A181T/V, D230G) are included in the LDA/EASL algorithm.

- Evivar SeqHepB resistance interpretations were identical when the mutation A181V was included, which according to the SeqHepB analysis confers intermediate resistance to LAMV (EFV, ADV, and TFV).

- The mutation T194S is detected by both the LDA and SeqHepB analysis. However, EASL does not include this mutation in its resistance interpretation algorithm. Other interpretation discrepancies included assignment of reduced sensitivity to tenofovir by SeqHepB for the resistance pattern L180M + N236T + V173L while EASL designates full resistance and the designation of reduced sensitivity by SeqHepB for the resistance pattern L180M + N236T + V173L while EASL designates full resistance and the designation of reduced sensitivity to tenofovir for the resistance pattern L180M + N236T + V173L while EASL designates full resistance and the designation of reduced sensitivity to tenofovir for the resistance pattern L180M + N236T + V173L while EASL designates full resistance.

- While resistance interpretations were generally concordant by the LDA/EASL and SeqHepB methods, this study shows that HBV sequence analysis results and reporting may vary based on which mutations are included and interpreted in a particular calling algorithm.

- The present study highlights the complexity of emerging HBV resistance patterns as well as the need for unified resistance calling algorithms.

REFERENCES:


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