EVALUATION OF IN-HOUSE REAL TIME PCR FOR HBV DNA QUANTIFICATION IN SERUM AND ORAL FLUID SAMPLES


BACKGROUND

Hepatitis B virus DNA (HBV DNA) quantitation in plasma or serum samples is a standard measure to start and monitor efficacy of treatment for people chronically infected with HBV, since sustainable suppression of HBV replication is one of the end points (EASL, 2017). Commercial molecular assays are used, but present high cost per reaction being difficult to be carried out in laboratories with low financial conditions and limited infrastructure, as well as in public health laboratories in the country that performs molecular diagnosis of HBV infection. Furthermore, some studies have shown that HBV DNA can be detected or quantified in oral fluid samples (Heiberg et al, 2010; Portilho et al., 2017) and that the blood collection and processing present difficulties in remote regions and some high-risk populations. Oral fluid samples could be an alternative to blood due to the ease of obtaining in individuals, especially children, elderly people, drug users and hemodialysis patients.

OBJECTIVES

The objective of this study is to optimize in-house real time PCR that uses a synthetic standard curve for HBV DNA quantification using serum and oral fluid samples to improve access to HBV diagnosis.

METHODS

SERUM AND ORAL FLUID SAMPLES

Samples and oral fluid samples were collected from 103 individuals (55 HBsAg positive and 48 without HBsAg markers in serum). Oral fluid samples were collected with Salivette device.

COMMERCIAL TESTS

Serum samples were submitted to serological tests and commercial PCR assay for HBV (Cobas TaqMan HBV, Roche) following manufacturer’s instructions.

IN-HOUSE REAL TIME PCR ASSAYS

Serum and oral fluid were tested using two in-house real time PCR assays for HBV pre-S/S region with different standard curves: qPCR using plasmidial curve and qPCR using synthetic curve (82 bp) (IDT® (CoralVille, USA). Analytical performance of qPCR using synthetic curve was evaluated and standardized prior to samples analysis:

- Analytical sensitivity and linear dynamic range
- Analytical specificity
- Reproducibility and repeatability
- Intra-assay accuracy

RESULTS

The authors have no conflicts of interest to declare.

REFERENCES


CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

IN-HOUSE PCR CURVES

Plasmidial curve: 9 points (5 x 10^0 to 50 copies/µL), linear regression coefficient (R2) of 0.998, “slope” of -3.43 and efficiency of E=95.7% (Figure 1A).

Synthetic curve: Efficiency value of E=99% (slope=-3.342; r=0.996), including eight dilution points, (10^0 to 10^8 copies/mL) (Figure 1B).

STUDY POPULATION

A total of 103 individuals were included and most of them were female (55/103) and mean age was 38.51 ± 13.59 years. In this group, 55 were HBsAg and anti-HBc positive in serum, one serum sample was HBsAg positive alone and none of them were anti-HBs positive, 11 were HBcAg and anti-HBc IgM positive, and 42 were anti-HBe positive. All HBsAg positive serum samples had HBV DNA detected by Cobas TaqMan HBV test.

EVALUATION OF PLASMIDIAL qPCR

Serum: 27 serum samples were HBV DNA positive showing mean viral load of 3.833 ± 1.822 log copies/mL (4.782 ≤ 1.878 log copies/mL, using Cobas TaqMan; r=0.8646; p<0.001; concordance of 72%) (Figure 2A).

Oral fluid: Five samples were HBV DNA positive (mean viral load of 4.073 ± 1.173 log copies/mL; r=0.1147; p=0.095).

EVALUATION OF SYNTHETIC qPCR

Serum: From 55 samples, 40 serum samples were positive by qPCR using synthetic curve with median of 2.947 log copies/mL and 3.972 log copies/mL by Cobas TaqMan; (r=0.9960; p<0.001; 85% of concordance) (Figure 2B).

Oral fluid: Ten samples were detected using qPCR with synthetic curve (mean viral load of 2.243 ± 1.607 log copies/mL; p=0.3671; r=0.3202).

CONCLUSIONS

This study demonstrated that the standardized qPCR with synthetic curve using serum samples can be used as alternative for HBV DNA detection and quantification, since it was reproducible, accurate, sensitive and significantly correlated to the results obtained by a commercial PCR method.

However, although in-house qPCR methods could detect HBV DNA in oral fluid samples, more tests must be done to improve sensibility.

Therefore, the development of in-house methodologies can decrease the costs of molecular diagnosis of HBV allowing the implementation of this technique in small laboratories with limited financial resources.

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