P0049
Paper Poster Session
Novel diagnostics for viral hepatitis

Semi-quantitative real-time PCR for hepatitis B diagnosis: a simple and cost-effective approach to identify and monitor low replicative chronic hepatitis B-infected patients in resource-limited countries

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Background: Antiviral therapy can be avoided during the low replicative phase of chronic Hepatitis B virus (HBV) infection, characterized notably by HBV DNA concentration below 2000 IU/ml, according to the last issues of WHO guidelines for HBV treatment. Simplified tests can improve access to HBV DNA monitoring in resource-limited settings. Here we evaluated performances of a semi-quantitative real-time PCR approach based on a single positive standard, to identify persons with low replicative hepatitis B infection.

Material/methods: One hundred and ten samples were selected from patients with chronic HBV infection. Twenty four control samples were also included to determine the specificity of the assay. HBV DNA levels were assessed using the semi-quantitative test on LightCycler 480 Instrument (Roche Diagnostics GmbH, Mannheim, Germany). DNA was extracted from 250 µl of serum with the Viral NA Extraction Kit (Biocentric, Bandol, France), using the Nordiag instrument (Biocentric, Bandol, France). The capacity of the semi-quantitative PCR to detect low replicative versus high/moderate HBV DNA samples was explored by comparing sample-to-standard CT ratio with HBV DNA levels obtained by the reference quantitative PCR test from Roche (CAP/CTM HBV Test v2.0).

Results:

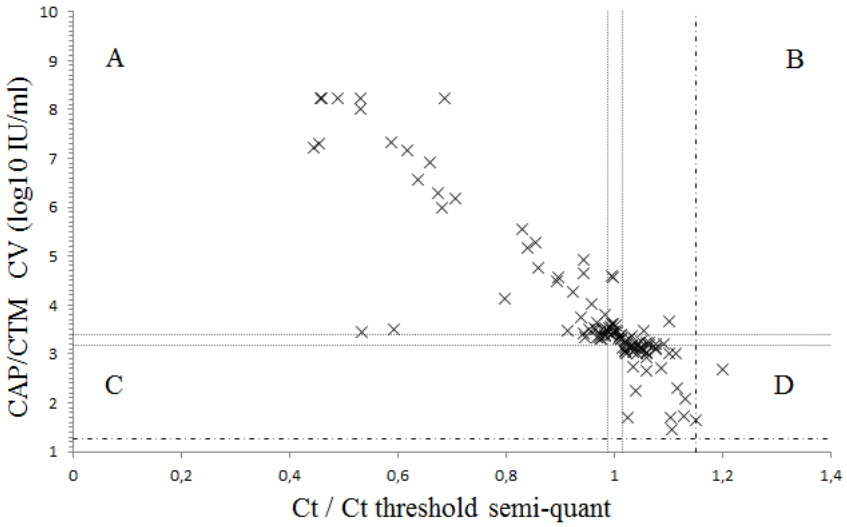

Figure 1: Correlation between the CAP/CTM test versus the semi-quantitative assay. Zone A: patient samples > 2000 HBV DNA IU/ml; zone B: patient samples < 2000 IU/ml with semi-quantitative assay and > 2000 IU/ml with the CAP/CTM test; zone C: patient samples > 2000 IU/ml with the semi-quantitative assay and < 2000 IU/ml with CAP/CTM test; zone D: patient samples < 2000 IU/ml The zone between solid lines defines the grey zone (i.e. between 1800 and 2200 IU/ml) while the dotted lines represent the LOD of both tests.

All samples tested positive, or negative, with the CAP/CTM test were also found positive, or negative, for HBV DNA using the semi-quantitative assay (sensitivity and specificity = 100%). The capacity of the semi-quantitative PCR to detect HBV DNA around the threshold of 2000 IU/ml was explored by comparing sample-to-standard CT ratio to HBV DNA levels (Figure 1). The semi-quantitative assay correctly identified all of the low replicative HBV DNA samples (<2000 IU/ml) and 58 out of 61 (95%) samples with moderate/high HBV DNA load compared to CAP/CTM test (>2000 IU/ml). The 3 misclassified samples had HBV DNA levels closed to the clinical threshold (<2000 IU/ml).

Conclusions: This study shows that the Universal HBV DNA PCR semi-quantitative HBV real-time PCR test has optimal clinical sensitivity when compared to a reference quantitative assay. The semi-quantitative approach is an alternative for diagnosing and following patients with HBV infection according to the threshold of 2000 IU/ml. This test is therefore compatible with a wide implementation and use in low-resource countries for improving access to hepatitis B viral load measurement.