POSTER REFERENCE NUMBER: 99

# STANDARDIZATION AND IMPLEMENTATION OF INTERNAL CONTROL IN HDV RT-QPCR ASSAY

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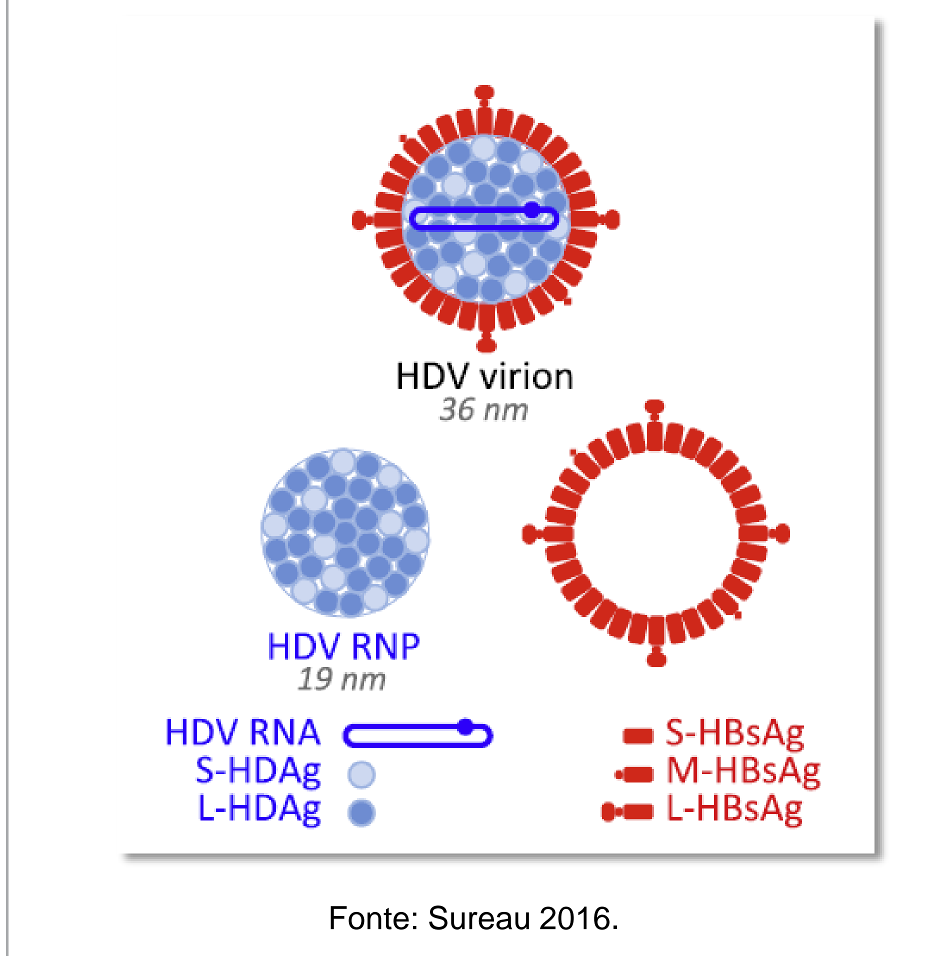
## BACKGROUND

The serological diagnosis for HDV infection is complex, due to the types of infections and the interpretation of the different serological markers. Currently, the polymerase chain reaction in real time quantitative (RT-qPCR) is often used to determine the viral load levels, due to its precision, sensitivity, specificity and reproducibility. The objective of this study evaluate the efficiency of the HDV RT-qPCR assay in the presence of internal control.

Figure 1. Hepatitis Delta Virus

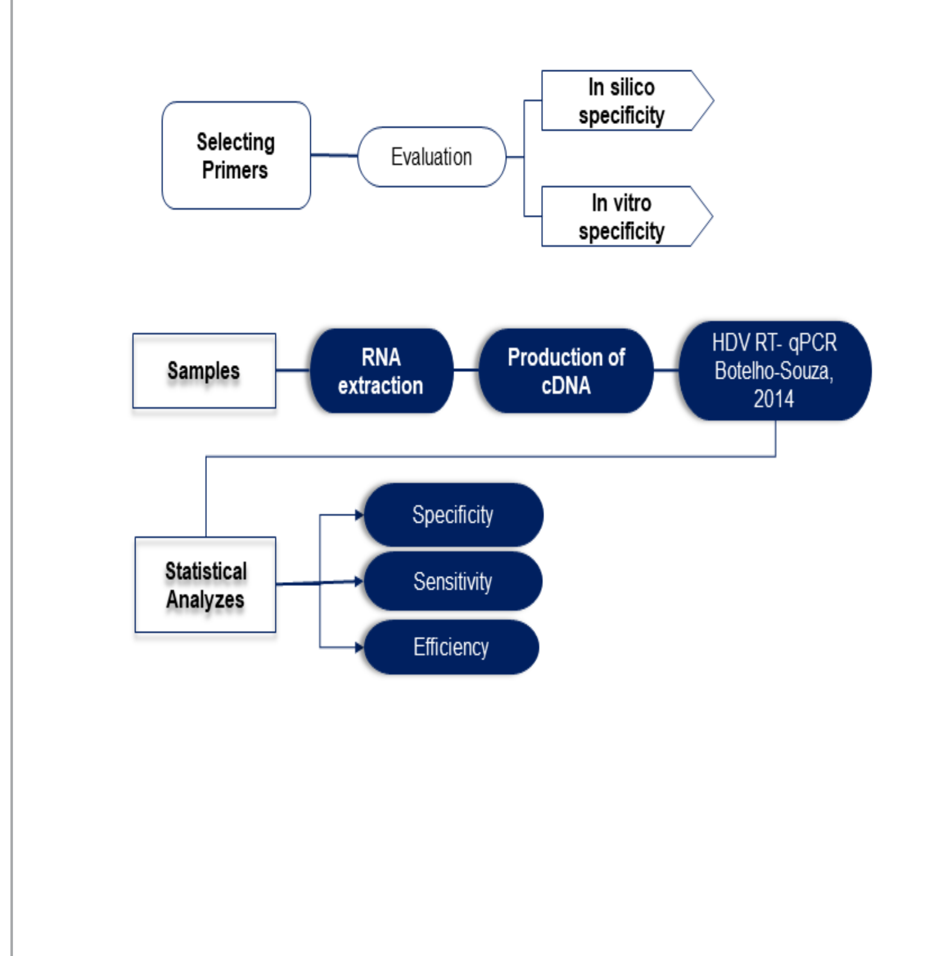



Fonte: Sureau 2016.

## METHODS

The methodology starts in the selection of the primers to verify the endogenous gene effective in the already standardized reaction. Then carry out in silico and in vitro evaluations to test the specificity and sensitivity of the primers. 100 samples of chronic HDV carriers are included in this study. The viral material will be extracted according to the manufacturer's recommendation by the Kit QIAGEN® RNA extraction, then cDNA production, for the insertion of the controls into the standardized reaction, generating a database for statistical tests, in order to verify the specificity, sensitivity and efficiency of the assay.

Figure 2. Molecular identification flowchart




## RESULTS

The results obtained are partial, where the specificity of the chosen primers was verified. The method used was the Sybr Green® system, observing the following results in the testing of the chosen concentration gradients of 300nM to 900nM, with amplification in the lowest concentrations tested. The dissociation curve showed a peak of 82° C in the β-actin gene and 77° C in the β-tubullin gene. Characterizing a specificity in the expected fragment.

Figure 3. Gradient Concentration β-actin (300nM to 900nM) and Dissociation Curve

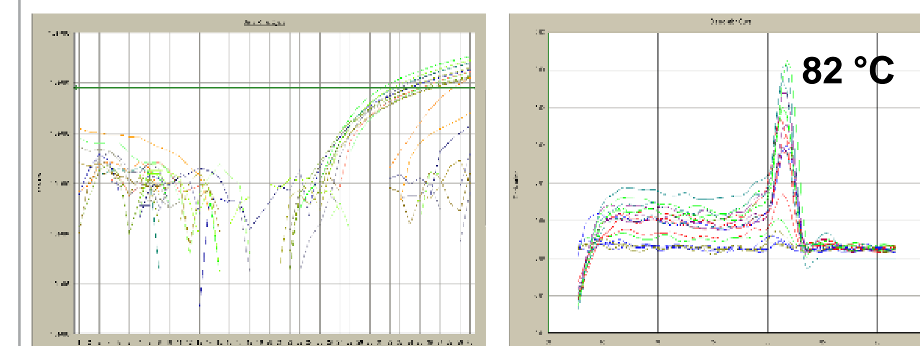
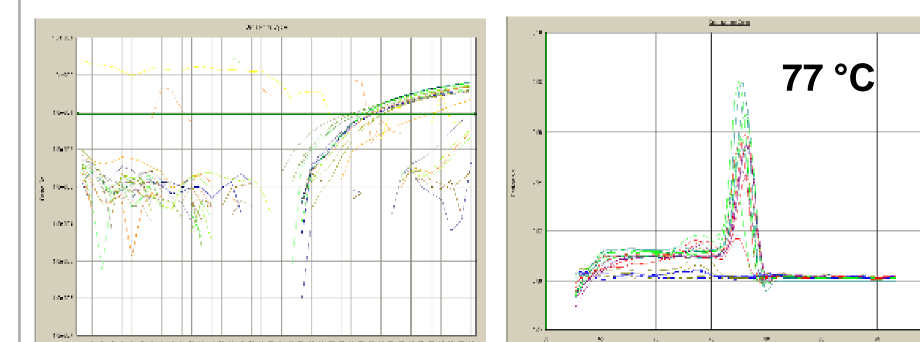



Figure 4. Gradient Concentration β - tubulin (300nM to 900nM) and Dissociation Curve




Amplification profile in Real Time PCR using Sybr Green detection system, respective dissociation temperatures are identified.

## CONCLUSIONS

In view of the above, the importance of internal control in the HDV RT-qPCR assay is verified, in order to reach its maximum analytical potential, by introducing appropriate standardization methods, helping to validate the results, making the diagnosis adequate and efficient, contributing to a better monitoring the clinical management of patients, enabling improvements in endemic areas, such as the Amazon region.

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## CONFLICTS OF INTEREST

There are no conflicts of interest between the authors of this study.

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