**AN ALTERNATIVE WAY FOR REAL TIME PCR DATA ANALYSIS**

**INTRODUCTION**
Real-Time PCR is a methodology with increasing applications in the clinical laboratory. This new and revolutionary method combines the PCR chemistry with the fluorescent probe/dye detection of the amplified product, all in the same reaction tube. Since the equipment used can record the emission of fluorescence during all the cycles of amplification, a significant increase of the PCR product is detected with the initial amount of target DNA. In Real-Time PCR, we can determine a fixed fluorescence threshold, above the background. When the PCR product that we want to detect crosses this threshold, we can determine a parameter named Cycle Threshold (Ct). All the equipment used in Real-Time PCR experiments have some kind of software to analyze the data, namely the analysis of the expression of Ct values relative to the logDNA. However, this software doesn’t give much details regarding the linear regression: it only calculates the Slope, Y intercept and Coefficient of Determination (R²). As an accredited laboratory, we have to monitor the performance of our methods. In Clinical Chemistry, the performance is measured with the determination of Total Error (TE) and Six Sigma (6σ), but until now it was never applied to Real-Time PCR.

**AIM**
Development of an Excel sheet that calculates several parameters regarding the linear regression and assay validation/calibration. Use of the data generated by this Excel sheet to monitor the performance of the In House Assays.

**METHODOLOGY**
We have implemented the Viral Load assays, using In House technology, for the quantification of Cytomegalovirus (CMV), Virus Epstein-Barr (EBV), Human Herpes Virus Type 6 (HHV6), Parovirus B19 (B19) and BK Virus (BKV). This techniques were described elsewhere (2, 3, 24). The methodology for the determination of Detection and Quantification Limits were described by Schwarz et al, 2004.

**RESULTS**
We have developed an Excel sheet that uses the data from calibrators (4 different concentrations of Virus) in order to determine the following parameters: Slope, Y Intercept, Coefficient of Determination (R²), Efficiency Amplification (E), Detection and quantification limit (analytical and method), Standard Error (RMSE) and P-Value associated with the linear regression (validation).

**CONCLUSION**
Our Excel sheet is a very good alternative to the data analysis of the standard software present in the various Real Time PCR equipments. It also allows the continuous monitoring of the data generated (internal quality control), together with the external Quality Assessment programs, can be used to monitor the performance of our In House assays.

**REFERENCES**