DIAGNOSIC PLATFORMS FOR DETECTION OF LATENT AND PERSISTENT VIRUSES (PARVOVIRUS B19 AND CYTOMEGALOVIRUS) IN HEMATOPOIETIC STEM CELLS PREPARED FOR TRANSPLANTATION AND TRANSPLANT RECIPIENTS

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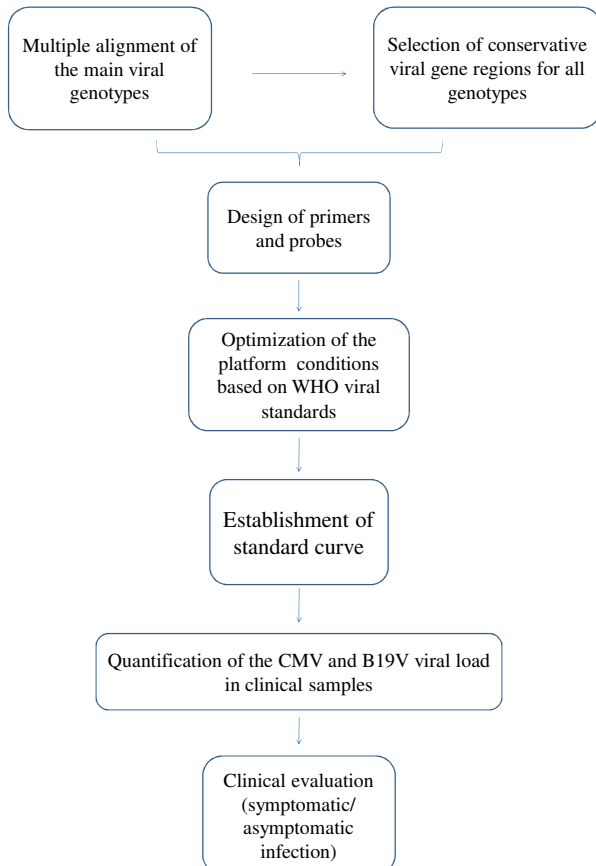
INTRODUCTION

Human parvovirus B19 (B19V) and cytomegalovirus (CMV) have significant tropism to hematopoietic stem cells (Sundin M. et al., 2008; Poole E. et al., 2011), where they establish persistent and latent infections. Moreover, both viruses are connected with severe clinical conditions among patients with hematopoietic grafts that can be often life-threatening. B19V is described as a causative agent of serious aplastic anemia and myelodisplastic syndrome (Urban C. et al., 2011) that can lead to delayed engraftment or prolonged poor graft function. Despite of all diagnostic and therapeutic advances, CMV continues to cause significant morbidity and mortality among transplant recipients. Pneumonia and gastrointestinal involvement are the most frequently described clinical conditions caused by CMV after transplantation. CMV pneumonia is a severe and often fatal complication despite the recent progress in its treatment. The severity of the gastrointestinal symptoms may vary depending on the location of the disease (Castagnola E. et al., 2004). Therefore, the prompt diagnosis of both viral agents in either hematopoietic stem cells or transplant recipients is of critical importance. In Brazil there is lack of diagnostic platforms for B19V and CMV due to their high price and legislative obstacles.

AIM

Our objective was to introduce and optimize real-time PCR TaqMan assays for B19V and CMV quantification and diagnosis.

EXPERIMENTAL STRATEGY

Multiple alignment of the main viral genotypes → Selection of conservative viral gene regions for all genotypes

Design of primers and probes

Optimization of the platform conditions based on WHO viral standards

Establishment of standard curve

Quantification of the CMV and B19V viral load in clinical samples

Clinical evaluation (symptomatic/ asymptomatic infection)

RESULTS

A) Optimization of UL97 real-time TaqMan PCR for quantitative detection of CMV. The optimized real-time PCR was in concordance with the NAT requirements for CMV detection: it had slope (-3. 467), Y-intercept (38.283), R^2 (0.999), and efficiency (97.704%). The linearity was excellent detecting CMV at seven orders of magnitude ($1-10^6$ copies/per reaction). The real-time PCR was optimized with the international WHO qualitative standard for CMV detection (code 09/162, NIBSC, UK) (**Figure 1**). The limit of detection was 1 copy/reaction.
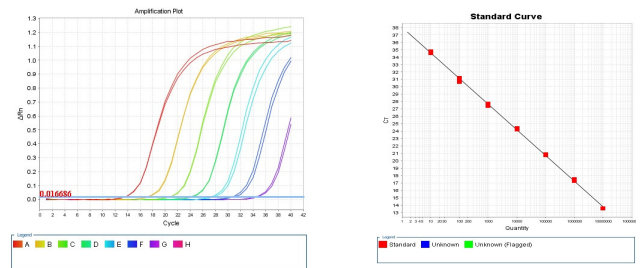

Figure 1. Amplification plot of the standard dilutions of the quantitative curve ($1-10^6$ copies/reaction) for CMV viral load determination. The efficiency of the amplification was fine (97.704%) and the curve was in wide dynamic range with limit of detection one viral copy per reaction. This fulfils the NAT requirements for quantitative CMV detection. Linear plot of the standard CMV detection curve and the plotted quantitative standards are demonstrated.

B) Optimization of VP1 real-time TaqMan PCR for quantitative detection of B19V. The optimized B19V VP1 TaqMan real-time PCR was in concordance with the requirements for B19V detection: slope (-3. 326), Y-intercept (39.825), R^2 (0.999), and efficiency (98.822%). The linearity was excellent detecting B19V at eight orders of magnitude ($10-10^7$ copies/reaction). The real-time PCR was optimized together with the international WHO standard for B19V detection (99/802, NIBSC, UK) (**Figure 1**). The detection limit was 10 copies/reaction.
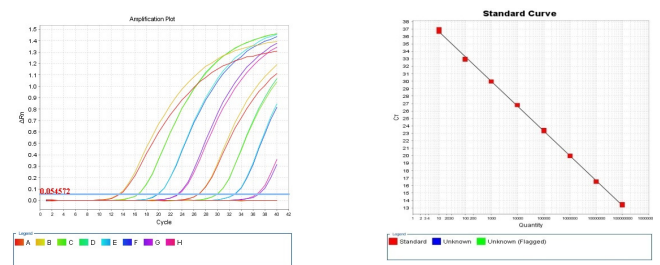

Figure 2. Amplification plot of the standard dilutions of the quantitative curve ($10-10^7$ copies/reaction) for B19V quantification. The efficiency of the amplification was fine (98.822%) and the reaction had detection limit of 10 copies. This fulfils the NAT requirements for quantitative B19V detection. Linear plot of the standard B19V curve and quantification curve with the plotted quantitative standards.

CONCLUSIONS

1. *Real-time TaqMan PCR is effective and rapid tool for B19V and CMV detection and quantification.*
2. *The quantification can be performed in a wide range of clinical samples.*
3. *The testing of the hematopoietic stem cells prepared for transplantation and transplant recipients is helpful for the further management of the patients.*

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