The prompt diagnosis of plasmodial species for correct and effective patient treatment prevents transmission, reintroduction of malaria and the worsening of health condition of the patient. The PCR allows detecting and quantifying parasites below the detection threshold of microscopic examination. The PCR method for *P. vivax* detection standardized in our laboratory is effective for detecting infection but does not allow the quantification and a diagnosis as fast as the real time PCR format. Furthermore, its precision, comprising the repeatability and reproducibility parameters, is unknown. Thus, our aim was to develop a real-time PCR assay with SYBR® Green and TaqMan® systems for the diagnosis of *P. vivax* malarial infection. Our experimental design included the construction of a standard curve with *P. vivax* DNA, cloned or not, to determine linearity; the setting of the lower detection limit and analytical sensitivity to measure sensitivity and; intra assay variations (repeatability) and oscillations between assays, operators and equipment (reproducibility) to set precision. The performance of these parameters showed linearity of $4 \times 10^4$ to 4 copies/µL with cloned DNA and $1 \times 10^4$ to 1 parasite/µL with uncloned *P. vivax* DNA, quantification threshold of 1.77 and 0.94 and analytical sensitivity of 1.13 and 1.17 copies/µL for SYBR® Green and TaqMan® systems, respectively. When compared conventional PCR with real time one, the detection limit remained 0.00001 parasite/µL and the precision was maintained 100% with 0.1 parasite/µL in SYBR® Green and 1 parasite/µL with TaqMan® and conventional PCR. We conclude that the real-time PCR is the eligible methodology for the detection of *P. vivax* parasites, the TaqMan® system is the most indicated for quantitative assays and this methodology could be used to replace conventional PCR in reference laboratories for the diagnosis of vivax malaria.

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