Multiplex Real-Time PCR Detection of Five Known Human Plasmodium Species

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Introduction

Plasmodium is the causative agent of malaria, an infectious disease that is transmitted by female Anopheles mosquitoes and affects over 200 million people worldwide. The disease is caused by five species of Plasmodium: Plasmodium vivax, Plasmodium falciparum, Plasmodium ovale, Plasmodium knowlesi, and Plasmodium malariae. These species can cause a variety of clinical presentations, ranging from mild to severe disease, and can result in lifelong infections. The diagnosis of malaria is crucial for effective treatment and prevention, and real-time PCR has become a valuable tool for detecting and identifying Plasmodium species. This study describes the development and validation of a multiplex real-time PCR assay for the simultaneous detection of five species of Plasmodium, which can provide rapid and accurate results.

Materials and Methods

Real-time PCR:

Total nucleic acid was isolated from blood samples using the QIAamp DNA Blood Mini Kit (Qiagen) and eluted in 50 μL of water. A non-competitive internal RNA control was added to the isolation buffer and used to monitor RNA extraction and real-time PCR efficiency. Amplification was performed using a set of primers and species-specific fluorescent probes targeting the 18S rRNA gene of P. falciparum and P. vivax. The assay was capable of detecting down to 100 copies of each species in whole blood, and it was able to distinguish between the five species of Plasmodium using conserved primers and species-specific fluorescent probes targeting the 18S rRNA gene. The assay was highly sensitive and had a rapid turn around of only a few hours compared to traditional methods.

Results

The assay was validated using positive patient blood and plasmid standards for all five species. Standard curves were generated with plasmids for all five species, and the assay was able to detect multiple species of Plasmodium using conserved primers and species-specific fluorescent probes targeting the 18S rRNA gene. The assay was able to detect as few as 100 copies of each species in whole blood, and it was able to distinguish between the five species of Plasmodium using conserved primers and species-specific fluorescent probes targeting the 18S rRNA gene.

Figure 1: Analytical Sensitivity and Standard Curve Determination

Figure 2: Co-Infection Sensitivity

Figure 3: Analytical Specificity

Figure 4: Plasmodium species variants

Conclusion

We have developed an assay capable of detecting all five species of Plasmodium in whole blood, which simplifies and speeds up the diagnostic process. The assay is highly sensitive and has a rapid turn around of only a few hours compared to traditional methods. This assay is an important tool in the detection and identification of Plasmodium species, which can help in the development of effective treatment strategies and prevention programs. It is important to note that a positive blood smear for malaria diagnosis is currently recommended for diagnosis. As a complement to traditional identification of Plasmodium species in blood smears, real-time PCR testing offers rapid and accurate results.

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Figure 5: Plasmodium species variants