中国寄生虫学与寄生虫病杂志 1999 17 (2): 109-112 ISSN: 1000-7423 CN: 31-1248/R

实验研究

# 疟疾复合PCR检测系统的建立

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收稿日期 修回日期 网络版发布日期 接受日期

摘要

目的：建立简易、快速的复合ＰＣＲ系统，用于检测间日疟、恶性疟及混合感染。方法：以疟原虫小亚单位核糖体核糖核酸基因为靶片段，设计疟原虫属特异性上游引物Ｓ１和间日疟原虫、恶性疟原虫种特异性下游引物Ｓ２和Ｓ３，建立双温度点复合ＰＣＲ扩增系统并用于临床血样的检测。结果：从间日疟原虫和恶性疟原虫感染血样中分别扩增出７０５ｂｐ和５７５ｂｐ特定扩增带，而食蟹猴疟原虫、诺氏疟原虫及健康人血样均未见扩增带。检测原虫水平达２－１０虫/μｌ全血。限制性内切酶酶切分析证实扩增产物为目的片段。检测１０４份镜检确诊疟疾患者血样，其中８１份与镜检结果相符，并查出镜检未发现的１７份混合感染和２份虫种鉴别失误的恶性疟。结论：本系统敏感性高，特异性强，操作简便，可在一次扩增中同时检出间日疟和恶性疟两种原虫。

关键词 PCR,间日疟原虫,恶性疟原虫,基因诊断

分类号



# ESTABLISHMENT OF A MULTIPLEX PCR SYSTEM TO DETECT PLASMODIUM *

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Abstract

AIM: To establish a simple, rapid and practical multiplex PCR system to detect Plasmodium vivax(P.v) and Plasmodium falciparum(P.f). METHODS: A common upper primer S1 and two species specific lower primers of P.v and P.f, S2, S3 were designed according to the sequences of the small subunit ribosomal DNA(SSUrDNA )fragments of the two Plasmodium species. Using these three oligonucleotide primers, the two temperature point multiplex PCR system was established and applied to detect P.v and P.f in the stock blood samples of clinically confirmed patients.RESULTS: DNA fragments of about 705 and 575 base pairs were successfully amplified by multiplex PCR from the genomic DNA of P.v and P.f, but no fragments were obtained from that of P.knowlesi, P.cynomolgi and blood of healthy persons. By means of restriction endonuclease digestion, the amplified fragments were confirmed to be the SSUrDNA fragments of P.v and P.f as expected. This method was successfully used in detecting parasitemia 2-10 parasite/μl whole blood. Of 104 samples tested by this system, 81 were coincident with microscopic examination. The multiplex PCR system also found 17 samlpes of mixed infection, which were not detected microscopically. Another 2 samples detected as P.v by microscopic examination were verified to be P.f infection by the multiplex PCR. CONCLUSION: The ease of operation together with high sensitivity and specificity, particularly the sensitive detection for mixed infection in a single run of amplification suggests that the multiplex PCR system might be a useful tool for malaria diagnosis.

Key words PCR Plasmodium vivax Plasmodium falciparum gene diagnosis

DOI:

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