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论著

标签引物-套式/多重PCR检测恶性疟原虫和间日疟原虫的研究

黄天谊1,王世海1,黎学铭2,郭传坤2,徐建军1,唐丽娜1,卢丽丹1

1 贵州省疾病预防控制中心， 贵阳 550001；
2 广西壮族自治区疾病预防控制中心， 南宁 530021

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摘要
目的 建立简便、灵敏、低本底、可同时检测恶性疟原虫和间日疟原虫的套式/多重聚合酶链反应（PCR）系统。 方法 应用标签引物扩增技术、Primer Premier 5.0软件、美国生物信息中心(NCBI-BLAST)网络资源和矩阵试验法优化套式/多重PCR，检测疟疾患者滤纸血样并与镜检结果进行比较。 结果 新建立的标签引物套式/多重PCR，检测模拟现场滤纸血样的敏感性为恶性疟原虫1～2个虫/μl血，间日疟原虫5～10个虫/μl血。检测71份现场采集的镜检疟原虫阳性滤纸血样（恶性疟24份和间日疟47份）的结果与镜检结果的符合率分别为87.5%和100%。 结论 通过标签引物扩增技术优化的套式/多重PCR系统，适用于检测现场采集的滤纸血样，其检出低原虫血症的敏感性和鉴定虫种的准确性均优于镜检法，是很有潜力的疟疾诊断技术。

关键词 恶性疟原虫 间日疟原虫 聚合酶链反应 标签引物 疟疾诊断
分类号

Tag Primer-Nested / Multiplex PCR for Detection of *Plasmodium falciparum* and *Plasmodium vivax*

HUANG Tian-yi,WANG Shi-hai,LI Xue-ming,GUO Chuan-kun,XU Jian-jun,TANG Li-na,LU Li-dan

Guizhou Provincial Center for Diseases Control and Prevention, Guiyang 550004, China

Abstract
Objective To establish a sensitive, simple to use and low noise nested/multiplex PCR for simultaneously detection of *Plasmodium falciparum* （P.f） and *Plasmodium vivax* （P.v）. Methods The tag primer amplification technique, software Primer Premier 5.0, NCBI-BLAST web resources and the matrix test were used to optimize the nested / multiplex PCR for detection of P.f and P.v with filter paper blood samples taken from malaria patients diagnosed by microscopy, and the results of the optimized nested/multiplex PCR and microscopy were evaluated. Results The sensitivity of the optimized PCR, determined by the examination of imitative filter paper blood samples, was about 1-2 parasites / μl for P.f and 5-10 parasites / μl for P.v. Primer-dimer and other PCR noise were removed. When 71 field filter paper blood samples taken from microscopically diagnosed patients (24 P.f, 47 P.v) were examined, the concordance between the optimized PCR and microscopy was 87.5% for P.f and 100% for P.v. Conclusion The nested/multiplex PCR optimized by tag primer amplification technique is simple, with low noise and being able to detect P.f and P.v simultaneously. It is more sensitive in detecting cases with low parasitaemia and more accurate in identifying *Plasmodium* species than microscopy.

Key words *Plasmodium falciparum* *Plasmodium vivax* PCR Tag primer Malaria diagnosis
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通讯作者
作者个人主页 黄天谊1;王世海1;黎学铭2;郭传坤2;徐建军1;唐丽娜1;卢丽丹1