中国寄生虫学与寄生虫病杂志 2007 25 (3): 213-216 ISSN: 1000-7423 CN: 31-1248/R

实验研究

套式/多重PCR诊断疟疾的敏感性、特异性和稳定性初探

郭传坤1,黎学铭1,李锦辉1,毛玮1,林珍1,杜进发1,黄天谊2

1 广西壮族自治区疾病预防控制中心, 南宁 530021;2 贵州省疾病预防控制中心, 贵阳 550004

收稿日期 修回日期 网络版发布日期 接受日期

摘要

【摘要】 目的 提高标签引物?鄄套式/多重PCR诊断疟疾的敏感性、特异性与稳定性。 方法 用滤纸法采集非疟疾发热病人血样30份及其他传染病（感冒， 流感， 伤寒， 肝炎等）患者血样20份；抽取恶性疟和间日疟各1例患者血4 m1，进行系列稀释制备不同疟原虫含量的感染血样；健康者血样作对照。用热裂解法制备DNA模板，用线粒体细胞色素氧化酶基因作为靶基因，应用相关软件和网络资源（Primer Premier 5.0, PUBMED, NCBI-BLAST和Mfold服务器）设计和优化标签引物?鄄套式/多重PCR，并用于检测所采集制备的各种血样。 结果 间日疟与恶性疟患者血系列稀释为含 1 000、100、10和1个虫/μl后经标签引物?鄄套式/多重PCR检测，恶性疟和间日疟患者各稀释含虫血样分别在611 bp和255 bp出现1条带，能检测到原虫密度均为1个虫/μl血；非疟疾发热病人血样30份及其他传染病患者血样均为阴性；健康者血样未出现扩增条带，每种血样反复测试3次以上均获得同样结果。 结论 经优化的标签引物-套式/多重PCR在疟疾诊断中具有较高的敏感性、特异性和稳定性。

关键词 恶性疟原虫 间日疟原虫 套式/多重PCR 标签引物 疟疾诊断

分类号

Sensitivity, Specificity and Stability of the Tag-primer Nested/multiplex PCR for Malaria Diagnosis

GUO Chuan-kun1,LI Xue-ming1,LI Jin-hui1,MAO Wei1, LIN Zhen1,DU Jin-fa1,HUANG Tian-yi2

1 Guangxi Center for Disease Control and Prevention，Nanning 530021, China; 2 Guizhou Provincial Center for Disease Control and Prevention，Guiyang 550001, China

Abstract

【Abstract】 Objective To improve the sensitivity, specificity and stability of the Tag-primer nested/multiplex PCR for malaria diagnosis. Methods Filter paper blood samples were collected from 30 non-malaria fever patients and 20 infectious disease patients （common cold, influenza, typhoid, hepatitis, etc.）. Four ml blood each taken from one falciparum malaria patient and one vivax malaria patient was serially diluted. Healthy blood sample was used as negative control. Improved direct heating method was used to prepare DNA template. The cytochrome oxidase gene （*coxI*） located in mitochondrion was selected as target gene. Relevant web resources and software （PUBMED, NCBI-BLAST, Mfold server and Primer Premier 5.0） were employed to design and optimize Tag-primer nested/multiplex PCR （UT-PCR） which was used to test all blood samples. Results A 611 bp band and a 255 bp band were seen in serially diluted infected blood samples （1 000， 100， 10 and 1 parasite/μl） from P.f and P.v patient tested by UT-PCR. The detection limit of either P.falciparum or P.vivax reached 1 parasite/μl, and the tested blood samples of non-malaria fever patients, patients with other infectious diseases and healthy persons were all negative. Consistent results of each sample in more than 3 duplicated tests were obtained. Conclusion The optimized Tag?鄄primer nested/multiplex PCR shows high sensitivity, specificity and stability in malaria diagnosis.

Key words *Plasmodium falciparum* *Plasmodium vivax* Nested/multiplex PCR Tag primer Malaria diagnosis

DOI:

通讯作者 黄天谊 tyhuang@163.com

作者个人主页 郭传坤1;黎学铭1;李锦辉1;毛玮1;林珍1;杜进发1;黄天谊2