中国寄生虫学与寄生虫病杂志 1999 17 (6): 380-383 ISSN: 1000-7423 CN: 31-1248/R

实验研究

复式PCR检测恶性疟原虫与间日疟原虫的研究

陈姝[1],陆惠民[1],高琪[2],唐学恒[1]

苏州医学院寄生虫学教研室,苏州 215007;江苏省寄生虫病防治研究所,无锡 214064

收稿日期 修回日期 网络版发布日期 接受日期

摘要

目的： 建立在同一次扩增中即可鉴别患者感染的疟原虫虫种的复式ＰＣＲ检测方法。方法： 根据恶性疟原虫（Ｐ．ｆ．）中度重复基因序列ｐＢＲＫ１－１４ 和间日疟原虫（Ｐ．ｖ．）线粒体细胞色素Ｃ氧化酶基因ＣＯＩＩＩ合成引物，采用经优化的ＰＣＲ反应体系， 对疟原虫ＤＮＡ模板进行扩增。结果： Ｐ．ｆ．与Ｐ．ｖ．分别被扩增出２０６ 和３７０ ｂｐ 大小的ＤＮＡ片段，与人白细胞ＤＮＡ无交叉；用该反应体系至少可检测出原虫血症为５×１０－ ７的Ｐ．ｆ．感染和１．０２×１０－ ６Ｐ．ｖ．感染； 自云南疟疾流行区采集的７８３份滤纸干血滴样本中， 复式ＰＣＲ法阳性检出率为８５．８％， 误诊率为０， 漏诊率为０．１％， 而镜检法依次分别为８４．９％ 、３．１％ 和１．０％， 两者符合率为９５．８％ 。结论： 本复式ＰＣＲ检测疟原虫较镜检敏感、特异， 适用于我国恶性疟与间日疟混合流行区的疟疾诊断、流行病学调查、药物的疗效考核和献血员的筛选等。

关键词 复式PCR 检测 恶性疟原虫 间日疟原虫

分类号

STUDIES ON DETECTION OF PLASMODIUM FALCIPARUM AND PLASMODIUM VIVAX IN BLOOD SAMPLES BY MULTIPLEX POLYMERASE CHAIN REACTION

CHEN Shu[1],LU Huimin[1],GAO Qi[2],TANG Xueheng[1]

1 Department of Parasitology;Suzhou Medical College;Suzhou 215007 2 Jiangsu Provincial Institute of Parasitic Diseases;Wuxi 214064

Abstract

AIM: To establish a sensitive and specific PCR-based method to detect Plasmodium falciparum and P.vivax in blood samples in a single amplification reaction. METHODS: Malaria parasite DNA in blood was amplified by the multiplex polymerase chain reaction using two sets of primers derived from the P.f. moderately-repetitive DNA sequence and COIII gene of P.v. RESULTS: A 206- bp product for P.f. and a 370- bp product for P.v. were amplified by multiplex PCR, being able to detect parasitemia level as low as 5×10\{-7\} for P.f. and 1.02×10\{-6\} for P.v. and having no cross-reaction with human leucocyte DNA. A total of 783 blood samples on the filter paper collected from patients attending to malaria clinics in malaria endemic areas were detected. The positive rate of multiplex PCR was 85.8%, the misdiagnosis rate was 0, and the under-diagnosis rate was 0.1%, while these three rates of microscopic examination were 84.9%, 3.1% and 1.0%, respectively. The concordance between the two methods was 95.8%. CONCLUSION: The multiplex PCR method made the malaria detection more sensitive and specific than the microscopic examination and should be suitable for the diagnosis of malaria in mixed endemic areas, large-scale epidemiological studies, follow-up of drug treatment and donor blood screenig.\;

Key words Multiplex PCR detection Plasmodium falciparum Plasmodium vivax

DOI:

通讯作者

作者个人主页 陈姝[1];陆惠民[1];高琪[2];唐学恒[1]