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### 巢式PCR法在疟疾检测及虫种鉴别中的应用

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#### Nested PCR for Malaria Detection and *Plasmodium* Species Identification

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**摘要** 根据疟原虫小亚单位核糖体核糖核酸 (SSU rRNA) 基因序列设计疟原虫通用型和种特异性的引物, 对60份血样进行巢式PCR检测及虫种鉴定, 并与血样的吉氏染色镜检结果进行比较。巢式PCR检出40份疟原虫阳性血样, 其中22份为恶性疟原虫 (*Plasmodium falciparum*) 阳性、13份为间日疟原虫 (*P. vivax*) 阳性、3份为恶性疟原虫和间日疟原虫混合感染、1份为卵形疟原虫阳性 (*P. ovale*)、1份未能分型。与镜检结果一致的血样为46份, 占76.7% (46/60), 其中恶性疟原虫阳性18份、间日疟原虫阳性11份和阴性17份。将两种检测结果不一致的血样进行扩增片段序列测定和实时荧光PCR分析, 检测结果均与巢式PCR结果一致。卵形疟原虫阳性血样扩增片段的序列分析结果显示, 该序列与卵形疟原虫SSU rRNA基因序列 (GenBank登录号DQ845247) 的对应部分同源率为100%, 证实该病例为输入性卵形疟原虫感染病例。

**关键词:** 疟疾; 巢式PCR; 小亚单位核糖体核糖核酸

**Abstract:** According to the sequences of small subunit ribosomal RNA (SSU rRNA) gene of *Plasmodium* spp., universal and species-specific primers were designed to detect malaria and identify species. 60 blood samples were detected by the established nested PCR method. The results were compared with those of microscopic examination. 40 blood samples were *Plasmodium*-positive by nested PCR with 22 samples of *P. falciparum*, 13 of *P. vivax*, 3 with *P. falciparum* and *P. vivax* mixed infection, 1 of *P. ovale* and 1 of unclassified malaria infection. Altogether, the coincidence between the results of nested PCR and microscopy stood for 76.7% (46/60), including 18 of *P. falciparum*, 11 of *P. vivax* and 17 negatives. Further sequence analysis and real-time PCR were performed to detect blood samples with discrepancy, results of which were the same as that of nested PCR. The amplified product of *P. ovale* was sequenced and showed 100% homology to the corresponding part of *P. ovale* SSU rRNA gene sequence (GenBank No. DQ845247), which confirmed that the case was imported *ovale* malaria.

**Keywords:** Malaria Nested PCR Small subunit ribosomal RNA

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