

| Nested PCR | Specificity of primers | Primer name ^Δ | Primer sequence ^Δ | Expected PCR product (bp) | Reaction con- ditions | Reaction sys- tems |
|--------------|------------------------|--------------------------|-----------------------------------------|---------------------------|-----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| First round | Genus | rPLU5 | 5'-CCTGTTGTTGCCT-TAAACTTC-3 ; | 1200 | 94°C for 3min;94°C for 30s, 58°C for 30s, 72°C for 60s, 34 cycles; 72°C for 5min. | 25 ul reaction volume in- cluding 2.6 μl template, 14.0 μl 2 × PCR Mix hybrid system (Containing Taq enzyme), 0.7 μl upstream primer (20umol / L) and 0.7 μl downstream primers (20umol / L) |
| | | rPLU6 | 5'-TTAAAATTGTTG-CAGTTAAAACG-3' | | | |
| Second round | <i>P.falciparum</i> | rFAL1 | 5'-TTAAACTGGT-TTGGGAAAAC-CAAATATATT-3' | 205 | 94°C for 3min;94°C for 30s, 60°C for 30s, 72°C for 60s, 34 cycles; 72°C for 5min | |
| | | rFAL2 | 5'-ACACAATGAACT-CAATCATGAC-TACCCGTC-3' | | | |
| | <i>P.vivax</i> | rVIV1 | 5'-CGCTTCTAGCT-TAATCCACATAACT-GATAC-3' | 120 | | |
| | | rVIV2 | 5'-ACTTCCAAGC-CGAAGCAAAGAAA-GTCCTTA-3' | | | |
| | <i>P.malariae</i> | rMAL1 | 5'-ATAACATAGTTG-TACGTTAAGAATAAC-CGC-3' | 141 | | |
| | | rMAL2 | 5'-AAAATTCCCATG-CATAAAAAATTATA-CAAA-3' | | | |
| | <i>P.ovale</i> | rOVA1 | 5'-ATCTCTTTTGCTAT-TTTTGTAGTATTGGA-GA-3' | 800 | | |
| | | rOVA2 | 5'-GGAAAGGA-CACATTAATTG-TATCCTAGTG-3' | | | |

[Snounou]: The primers' name and sequence were cited from References Snounou G, Viriyakosol S, Zhu XP, Jarra W, Pinheiro L, do Rosario VE, et al. High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. Mol BiochemParasitol. 1993;61(2):315-20.

Table1: The details of nested PCR testing for differentiating between various Plasmodium species.

Genetic confirmation by Yunnan Province Malaria Diagnosis Referent Laboratory

All of five imported-malaria cases were confirmed as mono-in-

fection by using Snounou's method in Yunnan Province Malaria Diagnosis Referent Laboratory. The results were as follows.

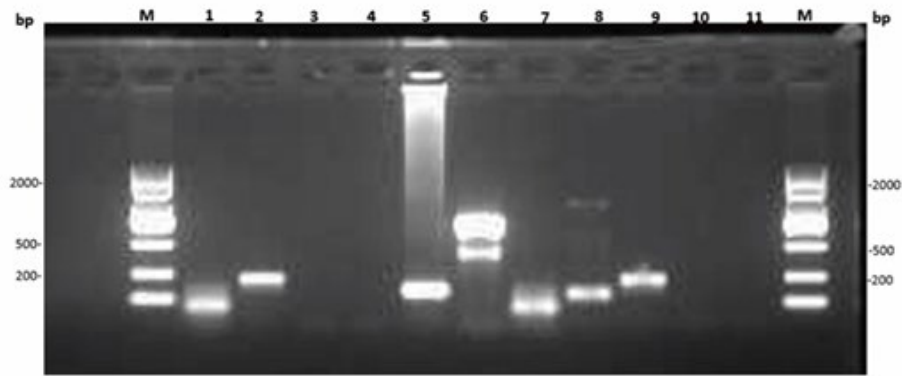


Figure 1: Electrophoretic image of the amplified products by nested polymerase chain reaction for five bone marrow fruit samples (1) M: DNA Marker; (2) 1 and 7: Case 1 and Case 5, *Plasmodium malariae*; (3) 2: Case 4, *Plasmodium falciparum*; (4) 5 and 8: Case 2 and Case 3, *Plasmodium vivax*; (5) 6: positive sample, *Plasmodium ovale*; (6) 9: positive sample, *Plasmodium falciparum*; (7) 3, 4: Negative sample; (8) 10 and 11: The blank controls of first-round PCR and second-round PCR, respectively.

| Patients | Infection source | Peripheral blood | | Bone marrow fruit | |
|----------|------------------|--------------------|------------------------|----------------------|-------------------|
| | | Species | Method | Species | Method |
| Case 1 | Congo | <i>P. malariae</i> | Microscopy examination | <i>P. malariae</i> | Genetic detection |
| Case 2 | Guinea | Negative | | <i>P. vivax</i> | |
| Case 3 | Cameroon | Negative | | <i>P. vivax</i> | |
| Case 4 | Guinea | Negative | | <i>P. falciparum</i> | |
| Case 5 | Congo | Negative | | <i>P. malariae</i> | |

Table 2: The information records of confirmation diagnosis for 5 malaria cases.