Table S1.PCR protocol and cycling conditions used for the malaria diagnostic for *P. falciparum*, *P. vivax,* *P. malariae* and *P. ovale*

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| --- | --- | --- | --- | --- | --- | --- |
|  | **Ingredients** | ***Pf*, *Pv*, *Pm*** | **Cycling conditions** | **Ingredients** | ***Po*** | **Cycling conditions** |
| **1st PCR** | Buffer | 2.5 µl | Initial denaturation: 94°C for 5 | Buffer | 2.5 µl | Initial denaturation: 94°C for 5 |
| dNTPs | 0.5 µl |  minutes | dNTPs | 0.5 µl |  minutes |
| 25µM primer 1st step Forward | 0.4 µl | Start cycle: 30 cycles | 25µM primer 1st step Forward | 0.4 µl | Start cycle: 30 cycles |
| 25µM primer 1st step Reverse | 0.4 µl | Denaturation: 94°C for 1 minute | 25µM primer 1st step Reverse | 0.4 µl | Denaturation: 94°C for 30 seconds |
| DNA template | 1 µl | Annealing: 60°C for 2 minutes | DNA template | 1 µl | Annealing: 45°C for 30 seconds |
| Taq Polymerase | 0.33 µl | Extension: 72°C for 2 minutes | Taq Polymerase | 0.33 µl | Extension: 72°C for 1 minute 30 |
| Water | 19.87 µl | End cycle | Water | 19.87 µl |  seconds |
| Total volume | 25 µl | Final extension: 72°C for 10 minutes | Total volume | 25 µl | End cycle |
| Final extension: 72°C for 10 minutes |
| **2nd PCR** | Buffer  | 2.5 µl | Initial denaturation: 94°C for 5  | Buffer | 2.5 µl | Initial denaturation: 94°C for 5  |
| dNTPs | 0.5 µl | minutes | dNTPs | 0.5 µl | minutes |
| PCR product of the 1st step | 3 µl | Start cycle: 30 cycles | PCR product of the 1st step | 3 µl | Start cycle: 45 cycles |
| 25µM primer (Pf) Forward  | 0.4 µl | Denaturation: 94°C for 1 minute | 25µM primer (Po) Forward | 0.4 µl | Denaturation: 94°C for 30 seconds |
| 25µM primer (Pf) Reverse  | 0.4 µl | Annealing: 55°C for 2 minutes | 25µM primer (Po) Reverse | 0.4 µl | Annealing: 45°C for 30 seconds |
| 25µM primer (Pv) Forward  | 0.4 µl | Extension: 72°C for 2 minutes | - | - | Extension: 72°C for 1 minute 30  |
| 25µM primer (Pv) Reverse  | 0.4 µl | End cycle | - | - | seconds |
| 25µM primer (Pm) Forward  | 0.4 µl | Final extension: 72°C for 10 minutes | - | - | End cycle |
| 25µM primer (Pm) Reverse  | 0.4 µl |  | - | - | Final extension: 72°C for 10  |
| Taq Polymerase | 0.33 µl |  | Taq Polymerase | 0.33 µl | minutes |
| Water | 16.27 µl |  | Water | 17.87 µl |  |
| Total volume | 25 µl |  | Total volume | 25 µl |  |