**Study of Antimalarial Drug Resistance Using the Muse® RBC Invasion Assay**

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**Introduction**

- The increasing resistance of *Plasmodium falciparum* to antimalarial drugs is a major concern for controlling the disease. The development of rapid methods—which can be used in a variety of lab environments—has become critical in the discovery of new antimalarial drugs.
- Using the compact and low-cost Guava® Muse® Cell Analyzer, an assay to evaluate the percentage of red blood cells (RBCs) invaded by *Plasmodium* parasites has been developed—the Muse® RBC Invasion Assay. The assay can be used for routine monitoring of RBCs invaded by different *Plasmodium falciparum* strains, as well as in vitro drug susceptibility studies.
- In this study, the in vitro inhibitory impact of chloroquine, mefloquine, and dihydroartemisinin on *P. falciparum* strains 3D7 and Dd2 was evaluated using the Muse RBC Invasion Assay. Significant differences in inhibition of these drugs on RBC invasion for the two strains were observed.
- The Muse RBC Invasion Assay can be a useful method for characterizing the in vitro impact of antimalarial drugs and a powerful drug development tool for malarial researchers.

**Results**

**Monitoring Malarial Cultures Using the Muse® RBC Invasion Assay**

Figure 2. Assessment of parasitemia in RBCs invaded by *P. falciparum* strains, 3D7, D10, and Dd2 are shown above (A). The Muse® RBC Invasion Assay offers simplified gating, and clear discrimination of populations, which is beneficial when applying the method to synchronization experiments. These synchronization experiments show a clear decrease of the trophozoites intraerythrocytic stage of the parasites, as shown in the 3D7 and Dd2 strains above (B).

**Evaluation of Multiple Strains in Antimalarial Drug Discovery**

Figure 3. The Muse® RBC Invasion Assay can be utilized to study the impact of antimalarial drugs on different *P. falciparum* strains. In the example above, RBC invasion with 3D7 and Dd2 strains was studied with different doses of antimalarial compounds—chloroquine and mefloquine—for 43 hours and 68 hours. Representative dot plots for Dd2-infected RBCs treated with chloroquine are shown (A). EC50 curves were mirrored for the results from both strains at 43 hours of treatment for chloroquine (B) and mefloquine (C), and summarized in Table C. The results show that while chloroquine has more potent antimalarial effects on the 3D7 strain, the Dd2 strain demonstrates significant resistance to treatment with chloroquine.

**Susceptibility of 3D7 and Dd2 to Dihydroartemisinin**

Figure 4. The Muse® RBC Invasion Assay was used to evaluate dihydroartemisinin, a more recent class of antimalarial artesimins. In the example above, RBCs infected with 3D7 (A) and Dd2 (B) strains were treated with dihydroartemisinin (DHA) at concentrations ranging from 0 to 400 nM for 43 and 68 hours prior to analysis with the Muse® RBC Invasion Assay. The results demonstrate the potent antimalarial effect of DHA at low concentrations. Further, RBCs infected with 3D7 and Dd2 strains both showed similar susceptibility to the drug with no significant resistance for either of the strains. The data demonstrates that based on the comparative effects of chloroquine and mefloquine (Figure 3), dihydroartemisinin has a broader capability and value as an antimalarial drug.

**Materials and Methods**

- In this study, the \( \text{Trophicholztides} \) of parasites, as shown in the 3D7 and Dd2 strains above (B).
- Using the compact and low-cost Guava® Muse® Cell Analyzer, an assay to evaluate the percentage of red blood cells (RBCs) invaded by *Plasmodium* parasites has been developed—the Muse® RBC Invasion Assay. The assay can be used for routine monitoring of RBCs invaded by different *Plasmodium falciparum* strains, as well as in vitro drug susceptibility studies.
- In this study, the in vitro inhibitory impact of chloroquine, mefloquine, and dihydroartemisinin on *P. falciparum* strains 3D7 and Dd2 was evaluated using the Muse RBC Invasion Assay. Significant differences in inhibition of these drugs on RBC invasion for the two strains were observed.
- The Muse RBC Invasion Assay can be a useful method for characterizing the in vitro impact of antimalarial drugs and a powerful drug development tool for malarial researchers.

**Conclusions**

- The Muse® RBC Invasion Assay is a quick and easy solution for the determination of percentage of red blood cells invaded by different *Plasmodium* strains on the low-cost Guava® Muse® Cell Analyzer.
- The actions of three antimalarial drugs on RBC invasion by 3D7 and Dd2 strains were evaluated. Results demonstrate that while chloroquine and dihydroartemisinin show potent antimalarial action at low doses, the Dd2 strain shows antimalarial resistance to chloroquine, but not to dihydroartemisinin.
- The capability to evaluate and compare antimalarial drugs on a simplified platform provides expanded tools to malarial researchers studying mechanisms of action.

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