Introduction

Today, malarial research spans a wide variety of researchers, from labs using simplified technologies that are proximal to samples, to labs involved in large scale, complex studies using multiple flow cytometry platforms.

One of the major questions regarding samples relates to the type of Plasmodium infection. The Muse® Malaria P.f.-P.v. Detection Assay is a novel, bead-based immunoassay for the detection of Plasmodium falciparum, Plasmodium vivax, and mixed infection samples on the simple, low-cost Guava® Muse® Cell Analyzer utilizing a dedicated software module for simplified yes/no callouts.

In this study, we expanded the application of the assay to multiple flow cytometry platforms, ranging from microcapillary cytometry-based Guava® easyCyte® Systems to sheath-based systems like the Amnis® CellStream® Analyzer.

Our data supports broader application of the Muse Malaria Kit across multiple cytometry platforms and its ability to provide high sensitivity analysis in plate-based and tube-based formats.

Background

The Muse® Malaria P.f.-P.v. Detection Assay is a bead-based assay for malaria identification and Plasmodium typing on the Amnis® Muse® Cell Analyzer. The assay detects Plasmodium vivax LDH, falciparum LDH, and falciparum HRP2; negative samples show no shift of beads, while positive vivax or falciparum samples will show shifts of the respective bead population. In this study, we expand the application of the assay to multiple flow cytometry platforms, such as the microcapillary Guava® easyCyte® Systems and sheath-based systems, such as the Amnis® CellStream® Analyzer. The assay can be run on cytometers with a blue or green laser, with the beads separated in red fluorescence and detection of analyte binding by PE detection in the yellow channel. The capability of being able to run the assay on a broader range of cytometers will provide flexibility when higher throughput is required.

Results

High Sensitivity Detection and Typing of P. falciparum, P. vivax, and Mixed Infection in Whole Blood Samples on the Guava® Muse® System

Figure 1 The Muse® Malaria P.f.-P.v. Detection Assay is a bead-based assay for malaria identification and Plasmodium typing on the Amnis® Muse® Cell Analyzer. The assay detects Plasmodium vivax LDH, falciparum LDH, and falciparum HRP2; negative samples show no shift of beads, while positive vivax or falciparum samples will show shifts of the respective bead population. In this study, we expand the application of the assay to multiple flow cytometry platforms, such as the microcapillary Guava® easyCyte® Systems and sheath-based systems, such as the Amnis® CellStream® Analyzer. The assay can be run on cytometers with a blue or green laser, with the beads separated in red fluorescence and detection of analyte binding by PE detection in the yellow channel. The capability of being able to run the assay on a broader range of cytometers will provide flexibility when higher throughput is required.

Figure 2 The Muse® Malaria P.f.-P.v. Detection Assay can be used with blood samples and can indicate the presence or absence of Pf HRP2, Pf LDH, and Pv LDH antigens and allow for typing of samples as P. falciparum, P. vivax, or mixed sample types. Panel A shows representative plots for the different sample types, with microscopy results in red. Positive shifts indicate the presence of antigen in frozen blood samples. Detection sensitivity (Panel B) was determined through dilution studies and demonstrates high sensitivity of ≤5 parasites/µL, which is superior to both RDTs and microscopy. The assay allows for easy typing with cross-talk across a wide parasitemia range. The assay on each instrument provided the ability to type the parasite and establish the presence of HRP2, Pf, and Pv LDH antigens using the Muse Malaria P.f.-P.v. Detection Assay.

Figure 3 Performance of the Muse® Malaria P.f.-P.v. Detection Assay across multiple flow cytometers was investigated. Malarial antigens HRP2 (A), Pf LDH (B), or Pv LDH (C) in a range of 0.2-100 ng/mL were spiked into donor whole blood, prepared, and run on the Guava® Muse®, plate-based Guava® easyCyte® 12HT and easyCyte® BGR, or Amnis® CellStream® Instruments. As shown in the plots above, clear shifts of relevant antigens could be seen in all instruments and similar relative MFI responses across a wide dynamic range of antigen concentrations could be obtained, as shown by the plots. Exportable MFI data can further facilitate antigen concentration determination in blood samples by comparison to standard curves. The Muse Malaria P.f.-P.v. Detection Assay displays the capability to be run on multiple cytometers with ease.

Figure 4 Frozen blood samples from 20 donors, characterized by microscopy, were prepared using the Muse® Malaria P.f.-P.v. Detection Assay in either tubes or plates using similar protocols. Samples were acquired on the Guava® Muse® Cell Analyzer, Guava® easyCyte® 12HT, easyCyte® BGR, or Amnis® CellStream® Analyzer. As shown in the table above, all four instruments provided clear and equivalent identification of falciparum and vivax antigens with no cross-talk across a wide parasitemia range. The assay on each instrument provided the ability to type the parasite and establish the presence of HRP2, Pf, and Pv LDH antigens using the Muse Malaria P.f.-P.v. Detection Assay.

Conclusions

• Malarial research needs access to both tube-based and plate-based methods of sample analysis to meet increasing needs in different environments.

• The Muse Malaria P.f.-P.v. Detection Assay provides important simultaneous detection of Pf HRP2, Pf LDH, and Pv LDH antigens, which allows for typing and identification of P. falciparum, P. vivax, and mixed sample types. The assay provides high detection sensitivity to ≤5 parasites/µL, which is superior to both RDTs and microscopy.

• In this study, we ran the Muse Kit on multiple cytometry platforms and demonstrated utility in both plate- and tube-based analyses. Our results show high sensitivity for antigen detection over a wide dynamic range and capability for concentration prediction. Application to frozen blood sample analysis allows for easy typing with good sensitivity across all platforms studied.

• The availability of highly multiplexed malarial antigen detection kits that can work across multiple cytometry platforms can empower malarial researchers to obtain enriched information on their samples with a high degree of sensitivity and speed.

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