



Muse[®] Malaria *P.f.-P.v.* Detection User's Guide

Catalog MPA100101 (100 tests)

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

Application

The Muse® Malaria *P.f.-P.v.* Antigen Detection assay is a qualitative kit for the detection and typing of the *Plasmodium falciparum* and *Plasmodium vivax* antigens in a variety of samples including; human whole blood, *plasmodium*-infected RBC cultures, and *plasmodium* antigen standards. The assay uses a combination of well characterized antigens: histidine-rich protein 2 (HRP2), *Plasmodium falciparum* lactate dehydrogenase (Pf-LDH), and *Plasmodium vivax* lactate dehydrogenase (Pv-LDH) to detect and identify the type of malarial infection.¹⁻⁷

Samples are prepared using the Muse® Malaria Antigen Detection Kit and analyzed on the Muse® Cell Analyzer with the Muse® Malaria *P.f.-P.v.* Detection software module. The software provides positive/negative callouts for detection of the following antigens:

- *Plasmodium falciparum* HRP2
- *Plasmodium falciparum* LDH
- *Plasmodium vivax* LDH

Sufficient reagent is provided for the preparation and analysis of 100 tests.

Test Principle

Muse® Malaria *P.f.-P.v.* Antigen Detection Kit is a bead-based antigen capture and detection assay. Three bead populations are conjugated to antibodies for specific *Plasmodium* antigens (one to *falciparum* HRP2, *falciparum* LDH, and *vivax* LDH). Samples are lysed and subjected to binding by the bead mix, in combination with a negative reference bead to assist in gating. Samples are probed with biotinylated antigen-specific antibody detector sets. Streptavidin PE is added to amplify the signal on beads. Positive beads are shifted to the right (antigen response) and easily detected by comparing with a negative reference bead. The callouts are provided based on the minimum bead number and shift into the corresponding antigen region.

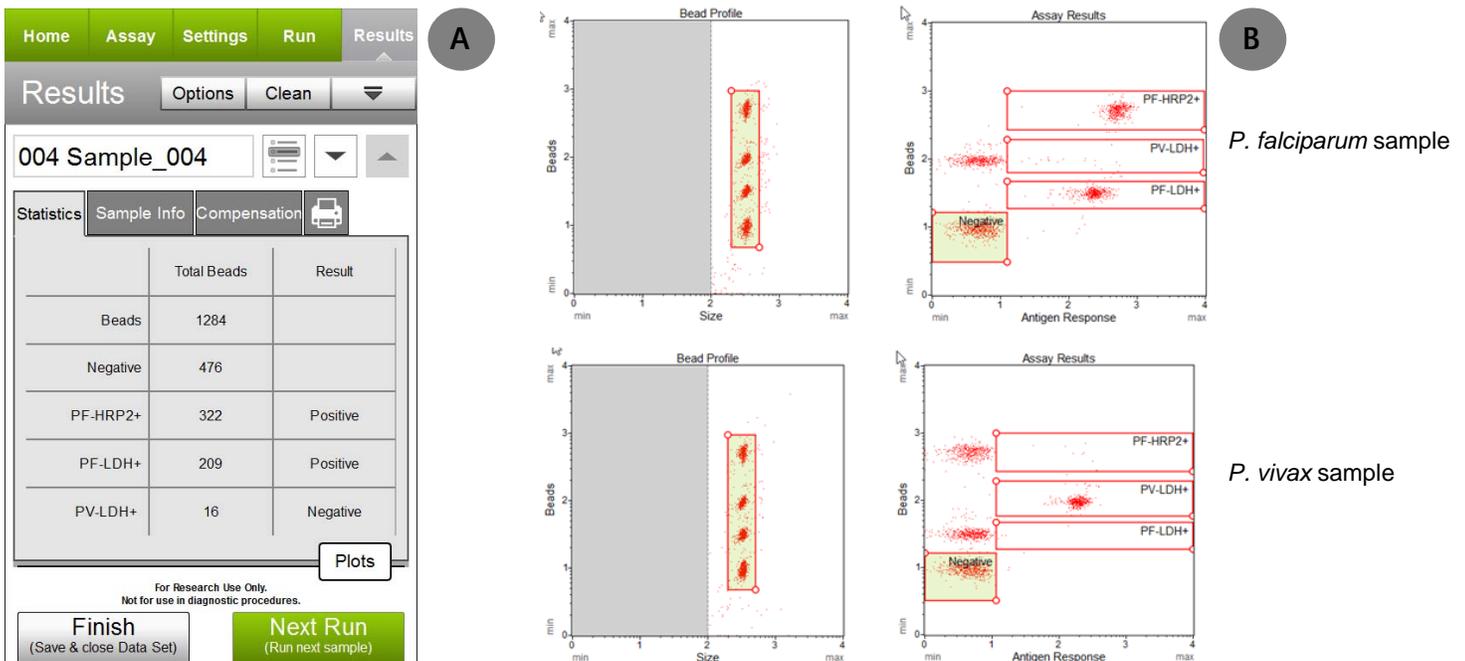


Figure 1. Example Data: Results from frozen whole blood stained with the Malaria *P.f.-P.v.* Detection assay and acquired on the Muse® Cell Analyzer using the Muse® Malaria *P.f.-P.v.* Detection software module. Figure A shows results without dot plots and Figure B shows the dot plots from a *P. falciparum* sample (top) and *P. vivax* sample (bottom). The statistics show the total number of beads detected in each population, as well as the corresponding result for PF-HRP2, PF-LDH, and PV-LDH. A positive result is defined by a shift of 200 or more beads into the gated region.

Summary of Protocol

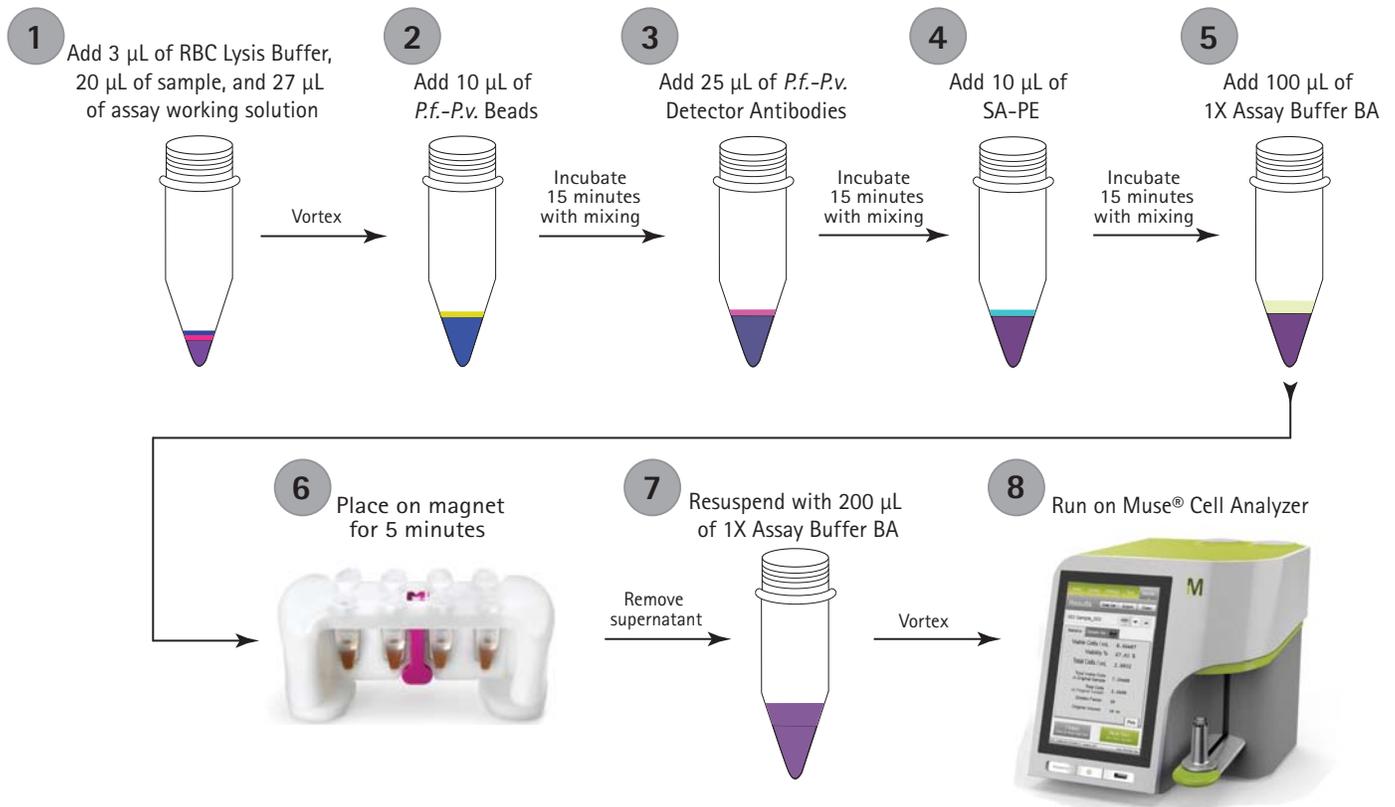


Figure 2. Summary of the protocol from sample preparation to analysis

Kit Components

Muse® Malaria *Pf.-P.v.* Antigen Detection Kit (Catalog No. MPA100101) contains the following reagents:

- 1X Assay Buffer BA (Part No. 4700-1360, 100 tests/vial)
- RBC Lysis Buffer (Part No. CS224562, 100 tests/vial)
- *Pf.-P.v.* Beads (Part No. CS224579, 100 tests/vial)
- *Pf.-P.v.* Detector Antibodies (Part No. CS224565, two vials, 100 tests/vial)
- Streptavidin-PE (Part No. CS224570 100 tests/vial)
- Blocking Buffer (Part No. CS224602, 100 tests/vial)

Materials Required but Not Supplied

- Muse® Cell Analyzer
- Micropipettes and disposable micropipette tips
- Microcentrifuge tubes with screw caps, 1.5 mL (VWR, Catalog No. 16466-030, or equivalent)
- Vortex mixer with tube adapter (example: Vortex-Genie® with 6" foam insert for 60 microcentrifuge tubes 504-0233-00, or equivalent)

- Magnetic rack (EMD Millipore™ LSKMAGS08, Dynal DynaMag™-2 Magnet12321D, Promega CD4002, or equivalent)
 - Disposable gloves
 - 100% bleach solution
 - Deionized (DI) water
 - Muse® System Check Kit (Catalog No. MCH100101)
 - Guava® ICF Instrument Cleaning Fluid (Catalog No. 4200-1040)
 - Positive control antigens or samples
-

Precautions

- The Muse® Malaria *P.f.-P.v.* Antigen Detection Kit is intended for research use only.
 - Avoid microbial contamination of the reagents, which may cause erroneous results.
 - All biological specimens and materials should be handled as if capable of transmitting infection and disposed of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Avoid specimen contact with skin and mucous membranes.
 - Wear proper laboratory attire (lab coat, gloves, safety glasses) when handling this reagent.
 - Exercise care to avoid cross-contamination of samples during all steps of this procedure, as this may lead to erroneous results.
 - The instructions provided have been designed to optimize the kit's performance. Deviation from the kit's instructions may result in suboptimal performance and may produce inaccurate data.
 - The fluorescent dyes in this reagent are light-sensitive. Store in the dark and shield from excessive exposure to light.
 - During storage and shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For maximum recovery of the product, centrifuge the vial briefly before removing the cap.
 - Do not use the reagents beyond the expiration date.
 - Material Safety Data Sheets (MSDS) for kit reagents are available from our website (www.emdmillipore.com/muse), by contacting EMD Millipore Technical Support, or from the EMD Millipore technical library at www.emdmillipore.com/techlibrary.
-

Storage

- Store the Muse® Malaria *P.f.-P.v.* Antigen Detection Assay Kit bag 1 of 2 refrigerated (2 to 8°C). Do not freeze.
- Store the Muse® Malaria *P.f.-P.v.* Antigen Detection Assay Kit bag 2 of 2 at -20°C.
- Return the Muse® Malaria *P.f.-P.v.* Antigen Detection Kit components to their appropriate storage conditions immediately after use. Do not leave out at higher temperatures for extended periods of time.
- The Muse® Malaria *P.f.-P.v.* Antigen Detection Kit contains light-sensitive dyes. Avoid prolonged exposure to light during storage and incubation.

Before You Begin

This protocol was developed for the detection of *P. falciparum* HPR2, *P. falciparum* LDH, and *P. vivax* LDH antigens in fresh or frozen whole blood, RBC cultures infected with malarial strains, and buffers spiked with antigens/standard proteins. We recommend the use of periodic positive and negative controls to ensure that the procedure and reagents are performing as expected.

Time considerations: The process of staining cells with the Muse® Malaria *P.f.-P.v.* Detection Kit takes approximately 1 hour. Acquiring data on the Muse® Cell Analyzer takes approximately 1 to 2 minutes per sample. Acquire samples within 24 hours after sample preparation is complete.

Always run a System Check prior to performing the assay. For details, refer to the *Muse® Cell Analyzer User's Guide*.

Assay Protocol

1. Remove reagents and controls (if applicable) from their appropriate storage conditions and allow them to come to room temperature.
2. Label one 1.5-mL microcentrifuge tube per sample or control with sample identification information, such as donor number.
3. Pipette 3 µL of RBC Lysis Buffer into the bottom of each sample tube.
NOTE: It is important to add the lysis buffer to the bottom of the tube to ensure proper lysing of the sample.
4. Reverse pipette 20 µL of well-mixed test sample into the bottom of each tube containing the RBC Lysis Buffer.
5. Create the assay working solution according to the table below. Cap the tube and mix well by vortexing at high intensity for 3 to 5 seconds.

Number of Tests	Blocking Solution	1X Assay Buffer BA
10	10 µL	290 µL
25	25 µL	725 µL

6. Add 27 µL of assay working solution to each tube. Cap the tubes and then pulse vortex each sample immediately at high intensity for 3 to 5 seconds.
7. Uncap the tubes and then add 10 µL of *P.f.-P.v.* Beads. Cap the tubes, place them in the tube adapter of the vortex, and cover with foil to protect from the light. Set the vortex on low speed and mix samples for 15 minutes at room temperature.
IMPORTANT: Ensure that the beads are mixed well by vortexing on high for 10 seconds prior to addition. The beads will settle so it is important to vortex the beads between each sample.
8. Add 25 µL of *P.f.-P.v.* Detector Antibodies to each sample tube. Cap the tubes, place them in the tube adapter of the vortex, and cover with foil to protect from the light. Set the vortex on low speed and mix samples for 15 minutes at room temperature.
9. Add 10 µL of SA-PE to each sample tube. Cap the tubes, place them in the tube adapter of the vortex, and cover with foil to protect from the light. Set the vortex on low speed and mix samples for 15 minutes at room temperature.
10. Add 100 µL of 1X Assay Buffer BA to each sample, briefly vortex and place in the magnetic tube holder for 3 to 5 minutes.

11. Carefully remove the supernatant using a 200 μ L pipette, taking care not to disturb the beads.

To remove the supernatant, depress the plunger on the pipette and carefully place the tip of the pipette down the front of the magnetic rack, taking care not to disturb the beads.

NOTE: The beads will remain on the backside of the tube towards the magnet. To minimize bead loss, place the pipette tip against the front side of the tube away from the magnet.



12. Immediately after removing the supernatant, remove the tubes from the magnetic rack, add 200 μ L of 1X Assay Buffer BA to each sample. Cap the tubes, mix, then place in the tube rack.

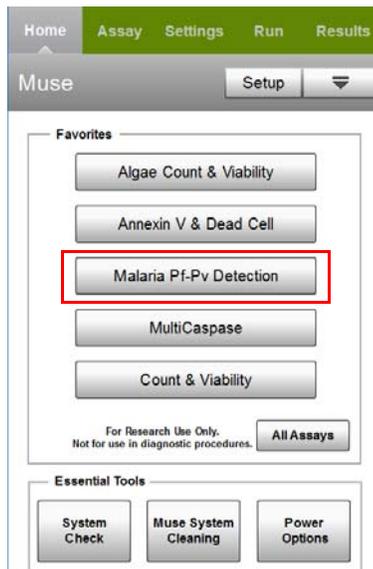
13. Acquire samples on the Muse[®] Cell Analyzer using the Muse[®] Malaria *P.f.-P.v.* Detection software module within 24 hours of sample preparation. Mix each samples well before loading it onto the instrument.

Setup and Acquisition on the Muse[®] Cell Analyzer

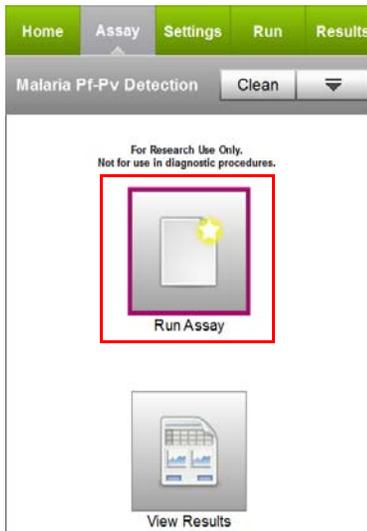
Run a System Check prior to performing the assay. For information on Muse[®] System Check, refer to the *Muse[®] Cell Analyzer User's Guide*.

1. Select the **Malaria Pf-Pv Detection** from the Favorites list.

If the assay does not appear in Favorites, select **All Assays**, then begin typing Malaria in the Filter field to display the assay, or select the **Other** category, then select **Malaria Pf-Pv Detection**.

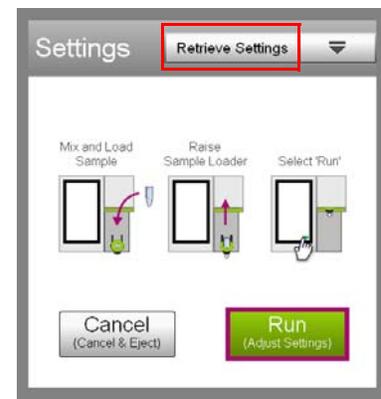


2. Select **Run Assay**.



3. Adjust the instrument settings.

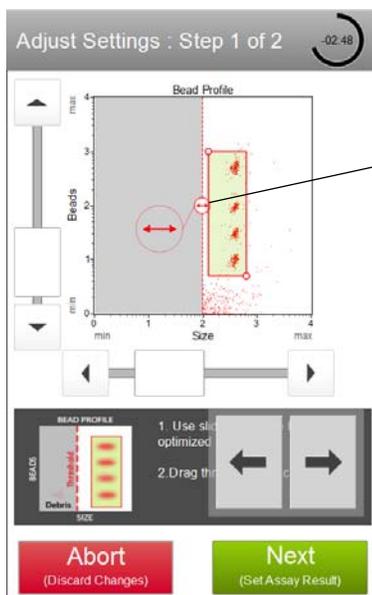
- Load a negative control sample to adjust the settings and select **Run** (Adjust Settings).
- Or, to retrieve previously saved instrument settings, select **Retrieve Settings**. For more information on retrieving settings, see the *Muse® Cell Analyzer User's Guide*.



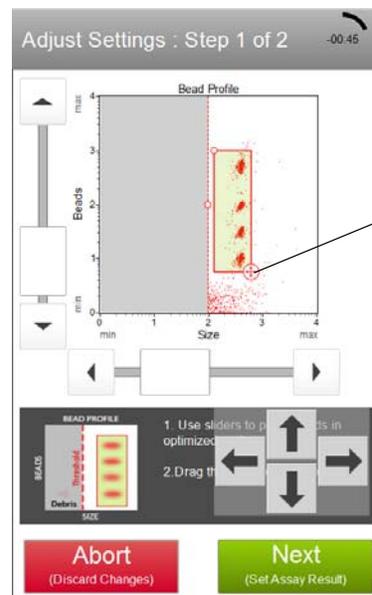
4. Fine tune the settings for the Size vs Beads plot, if necessary.

- Adjust the Size slider below the plot to move the bead population into the counting gate (green region).
- Adjust the gate to exclude debris.
- Adjust the threshold to exclude debris. Touch and drag the threshold to make large changes. Touch the arrow buttons below the plot to make small changes. The arrow buttons appear after you touch the threshold.

NOTE: If the acquisition times out (after 4 minutes), you can select **Back** to restart the adjust settings step, or **Next** to accept the settings and continue to the next step. If acquisition times out, remove the tube and mix well before restarting the adjust settings step.



Touch to adjust the threshold

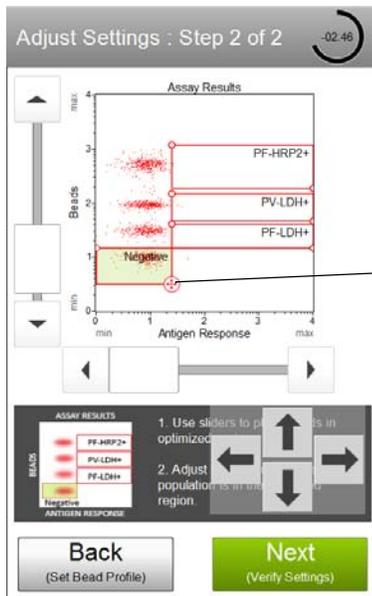


Touch to adjust the gate.

5. Select **Next** when you've completed the adjustments.
6. Fine tune the settings for the Antigen Response vs Beads plot, if necessary.
 - Adjust the vertical and horizontal sliders (to the left of and below the plot) to position the negative beads in the green region.
 - Adjust the gate around the negative bead population (green region) so that the right side is positioned tightly around the beads.

NOTE: The right side of the negative bead population is linked to the left side of the PF-HRP2, PV-LDH, and PF-LDH populations.

- Adjust the top and bottom of the PF-HRP2, PV-LDH, and PF-LDH regions so that the three bead populations are aligned with the region.

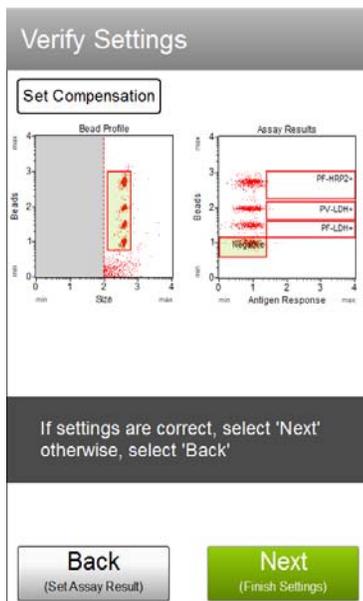


Touch to adjust regions.

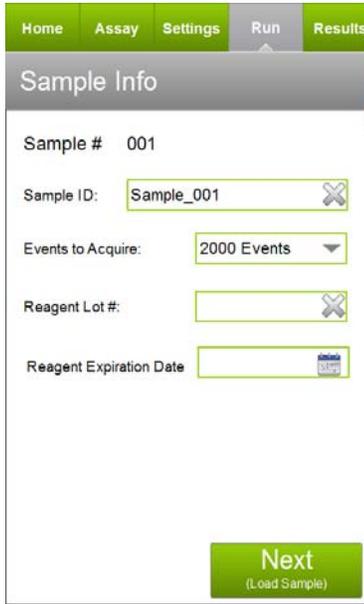
Adjust the upper and lower boundaries up or down.

The right side of the negative region and the left sides of the positive regions move together.

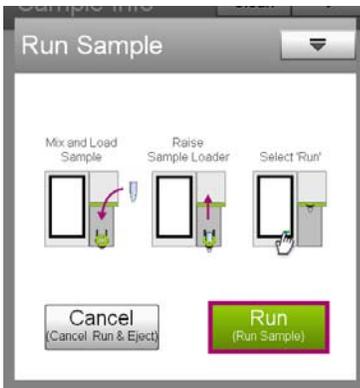
7. Select **Next** when the adjustments are complete.
8. Verify the settings. If the settings are correct, select **Next**. Otherwise, select **Back** and repeat steps 4 through 7, as necessary.



9. Enter the sample ID, reagent lot #, and reagent expiration date by touching the field, then using the keypad to input the value. Touch **Done** when you've finished entering the information. If necessary, change the Events to Acquire by touching the field, then selecting the value from the pop-up menu. Select **Next**.

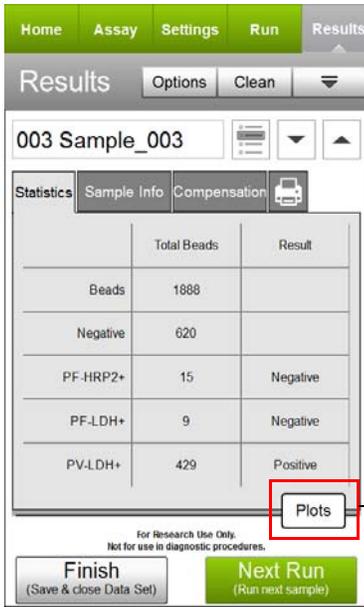


10. Mix the first sample and load it on the instrument. Select **Run** (Run Sample).

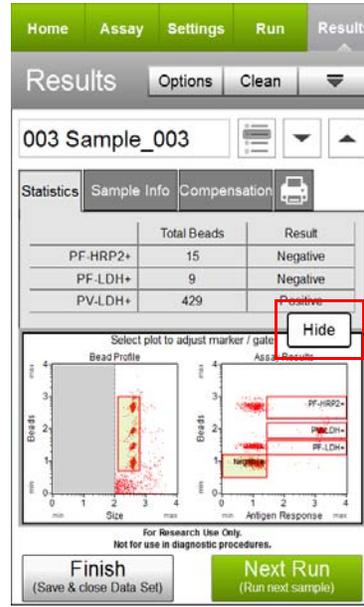


11. When acquisition is complete, the results are displayed. If necessary, select **Plots** to display dot plots for the sample.

You can view or change the sample ID, as well as add annotations for the current sample, by selecting the Sample Info tab. To print the results for the current sample select the printer tab.



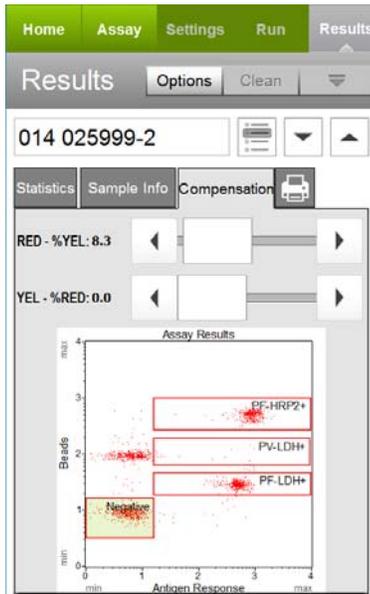
Select to display plots.



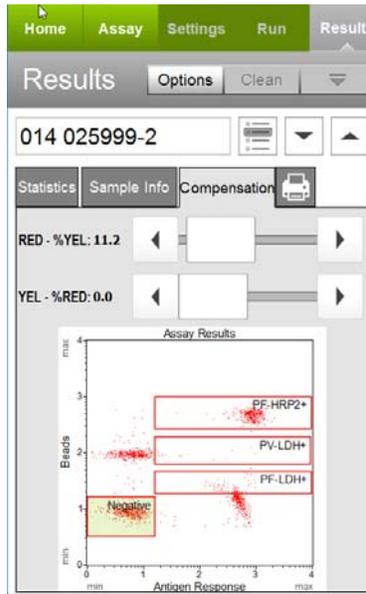
Select to hide plots.

- (Optional) If changes are needed to the gates, touch a plot to enlarge it, then adjust the bead profile gate and/or assay results markers/gates as described in steps 4 and 6. You cannot adjust the size threshold after the sample has been acquired.
- Compensation:** If you notice the beads bleeding from one region to the next, it may be necessary to adjust the compensation for RED-%YEL. By default, the compensation is set to RED-%YEL: 8.3. Click on the Compensation tab and adjust the slider using the arrows for fine adjustment or moving the bar for course adjustment. See the examples below.

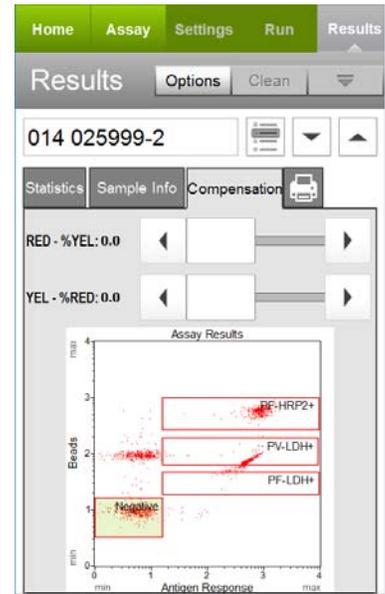
Correct Compensation



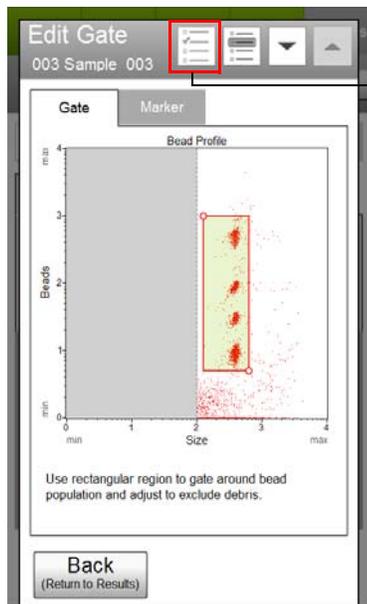
Over Compensated



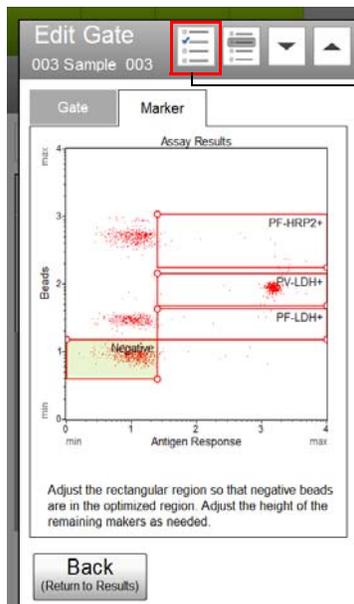
Under Compensated



14. If you adjust the gate, marker, or compensation and wish to apply the changes to other samples that you already acquired, select the Apply Changes button () in the title bar. Select the samples you want to apply the changes to or choose **Select All**, then select **Apply**. The sample you originally made changes to must be selected.



Apply changes



Select to apply changes to other samples.

15. If no adjustments are needed, select **Next Run** and repeat steps 9 through 12 for the remaining samples.

NOTE: During the run, a message may appear prompting you to load a tube of DI water for a Quick Clean. Load the water then select **Clean** to perform the Quick Clean. Select **Next** to continue with the run. The frequency of Quick Cleans was set by your system administrator. Your administrator may also have chosen to allow you to skip the Quick Clean when the prompt appears. You can choose to perform additional Quick Cleans at any time during a run by selecting **Clean** in the title bar, then **Quick Clean** from the menu.



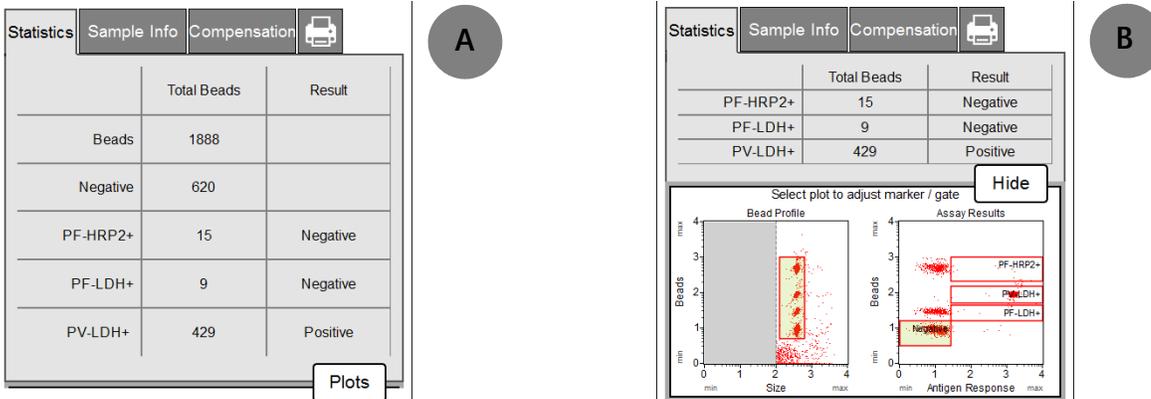
16. When you have acquired the last sample, select **Finish**.

17. (Optional) Select **Options** in the title bar to rename the data set, export the data set, save the current instrument settings, or view the event log. Refer to the *Muse® Cell Analyzer User's Guide* for more information.

Results

The Muse® Malaria *P.f.-P.v.* Antigen Detection software displays results on the screen after each sample is acquired. Results displayed include sample information, test results for presence/absence (positive/negative) of each malarial antigen, and the number of beads for each population. Results can be displayed without plots (A) or with dot plots (B).

You can export the report as a PDF file to a USB drive. Refer to the *Muse® Cell Analyzer User's Guide* for more information on printing.

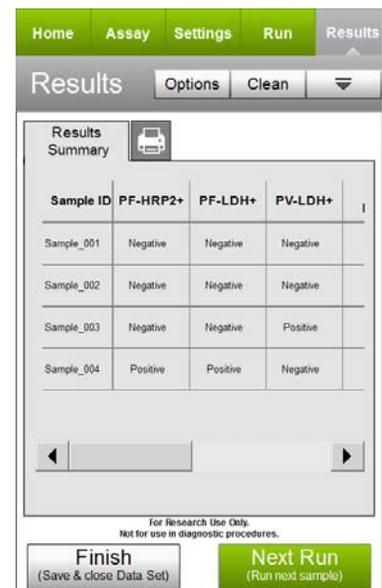
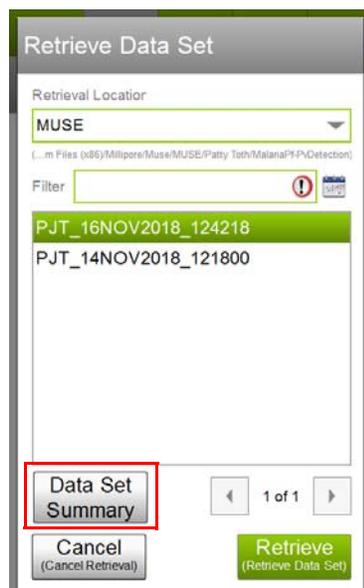
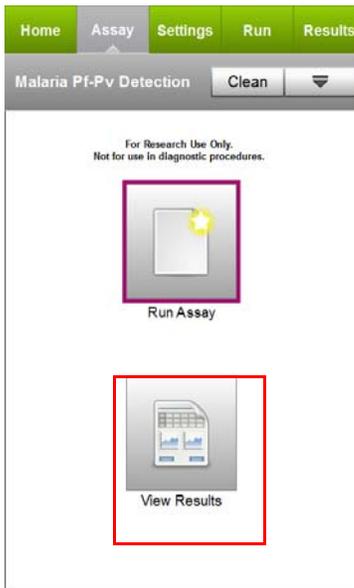


Results from each run are stored in a data file, as well as its corresponding spreadsheet (CSV) file. The spreadsheet file contains the following statistics:

- Sample number
- Sample ID
- Sample number
- Total number of beads collected
- Count, result, mean, and median intensity of the following populations:
 - Negative
 - PF-HRP2
 - PF-LDH
 - PV-LDH

Summary Report

You can print a data set summary sheet by selecting **View Results** from the Assay tab, selecting the data set, then selecting **Data Set Summary** from the Retrieve Data Set screen.



The data set summary contains the following information for each sample in the data set:

- Sample ID
- PF-HRP2+ result and count
- PF-LDH+ result and count
- PV-LDH+ result and count
- Total bead count
- Negative bead count

Example Data from Muse P.f/P.v Antigen Detection Assay:

The Muse® Malaria *P.f.-P.v.* Antigen Detection Assay is capable of detecting *P. falciparum*, *P. vivax*, and mixed infection antigens in fresh or frozen blood samples. Sensitivity is demonstrated using dilutions of *P. falciparum* and *P. vivax*, and FIND/Zeptomatrix culture panels.

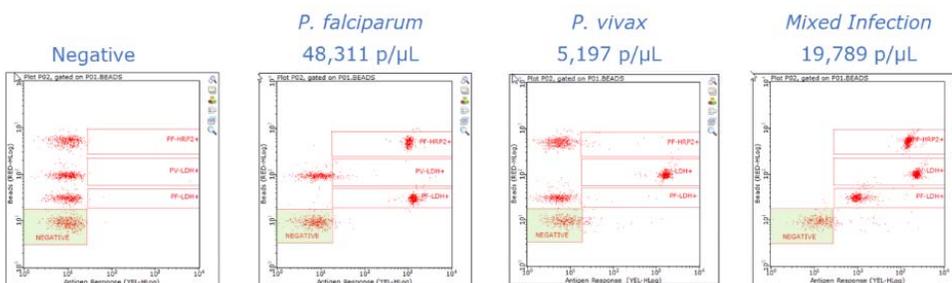


Figure 3. Example results from whole blood donors from Negative, *P. falciparum*, *P. vivax* and mixed infection samples. Parasitemia values were characterized by the vendor using standard microscopy techniques.

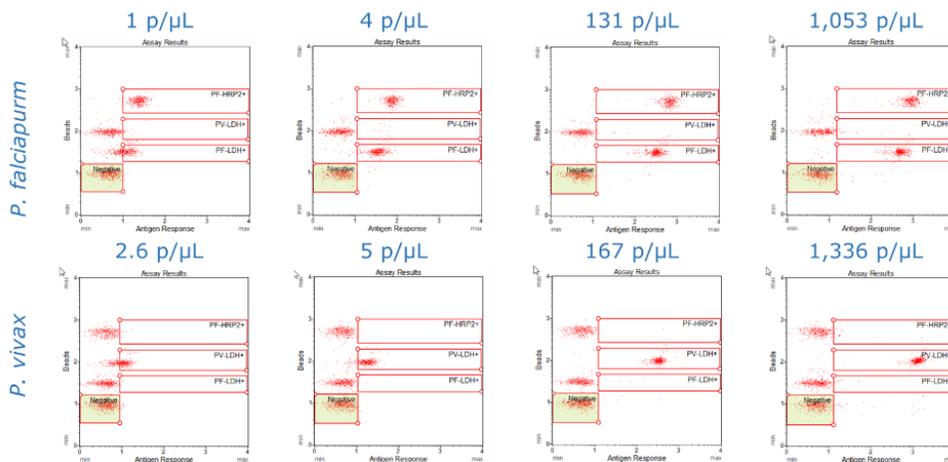


Figure 4. Sensitivity of the assay has been demonstrated using dilution studies of *P. falciparum* (top panel) and *P. vivax* (bottom panel) blood samples with known parasite count previously characterized by microscopy.

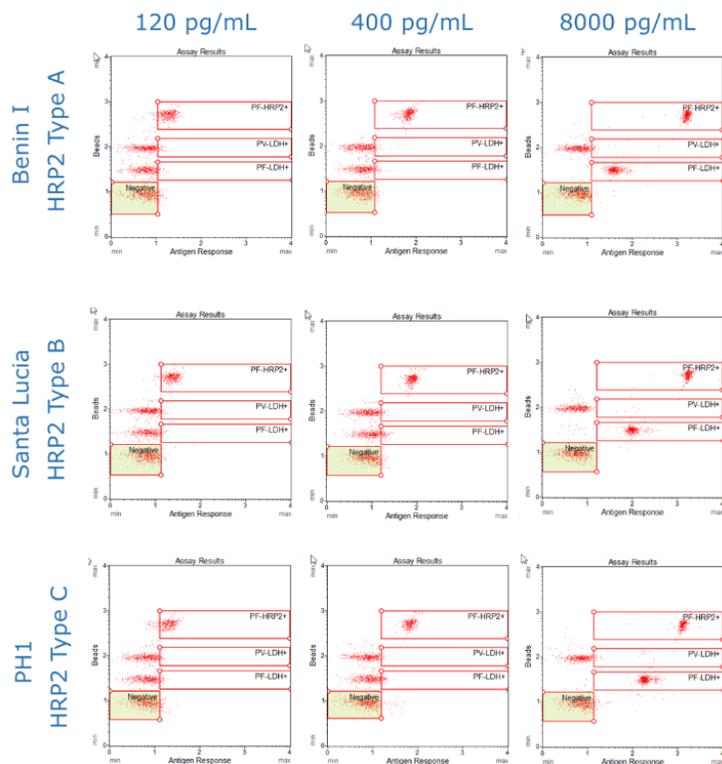


Figure 5. Assay performance with FIND/Zeptomatrix Culture Panels with known HRP2 concentrations. Results above are from Benin (top panel), Santa Lucia (middle panel) and PH1 (bottom panel).

Technical Tips

1. Careful, accurate pipetting with calibrated pipettors is required for accurate and consistent results in the Muse® Malaria *P.f.-P.v.* Antigen Detection assay. Mix cell samples well. Beads will settle to the bottom of the tube over a short period of time.
2. Allow all reagents to come to room temperature prior to starting.
3. For optimal assay performance ensure that the *P.f.-P.v.* Beads are well mixed prior to addition. It is also important to mix samples during each incubation to prevent beads from settling.
4. Use Guava ICF periodically to clear the fluid system of buildup from cell samples and reagent residue. After finishing the Malaria *P.f.-P.v.* Detection assay, run Quick Clean with Guava ICF followed by water to remove dye residue that could carry over to other assay tubes.
5. Compensation may need to be adjusted to bring the bead populations within their appropriate gates.

Troubleshooting

Potential Problem	Experimental Suggestions
Acquisition taking longer than expected or progress bar stops during acquisition	Ensure that the System Check procedure was run and passed. If the progress bar stops during acquisition, the fluid system may be clogged. Run a Quick Clean procedure.
Instrument clogging; too many events	Run a Quick Clean to clean out the capillary. This procedure can be performed during or after an assay.
<i>Low Bead Concentration</i> warning during acquisition	Handle samples carefully during separation steps to minimize bead loss during sample preparation. Insufficient beads will result in the software miscalling the population. Repeat sample preparation and testing. Vortex samples prior to running on the Muse® Cell Analyzer, as beads can settle quickly and impact the count.
Background staining and/or non-specific staining of samples	If samples have high background staining, the blocking step may need to be optimized or the sample may need to be diluted. Prepare sample again using several concentrations to ensure adequate blocking of interfering substances.
Low level of staining	To ensure the procedure is working properly, the use of positive and negative controls is recommended. If control staining is low, prepare your samples making sure that the correct volume of each reagent is added and that mixing is performed at each step.
Variability in day-to-day experiments	If there appears to be day-to-day variation of the staining pattern, ensure the Muse® Cell Analyzer is working properly. Run the Muse® System Check procedure (Catalog No. MCH100101) to verify proper instrument function and accuracy.

For more information, contact the EMD Millipore office nearest you. In the US, call 1-800-MILLIPORE (1-800-645-5476). Outside the US, visit our website at www.emdmillipore.com/offices for up-to-date worldwide contact information. You can also view the tech service page on our web site at www.emdmillipore.com/techservice.

Limitations

- The results of the assay are dependent upon proper use of reagents, products, and instruments.
- The software predicts results based on a minimum number of beads of each population observed (200 beads in a region defines a positive result).

Related Kits

- Muse® System Check Kit - MCH100101
- Guava® ICF (Catalog No. 4200-1040)
- Muse® RBC Invasion Kit (MMA100101)

References

1. Bousema T, Okell L, Felger I, Drakeley C. Asymptomatic malaria infections: detectability, transmissibility and public health relevance. *Nat Rev Microbiol.* 2014;12(12):833–840.
2. Jimenez A, Rees-Channer RR, Perera R, Gamboa D, Chiodini PL, González IJ, Mayor A, Ding XC. Analytical sensitivity of current best-in-class malaria rapid diagnostic tests. *Malar J.* 2017;2416(1):128. doi: 10.1186/s12936-017-1780-5.

3. Berhane A, Anderson K, Mihreteab S, Gresty K, Rogier E, Mohamed S, Hagos F, Embaye G, Chinorumba A, Zehaie A, Dowd S, Waters NC, Gatton ML, Udhayakumar V, Cheng Q, Cunningham J. Major Threat to Malaria Control Programs by Plasmodium falciparum Lacking Histidine-Rich Protein 2, Eritrea. *Emerg Infect Dis.* 2018;24(3):462–470. doi: 10.3201/eid2403.171723.
4. Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A, Wernsdorfer WH. A review of 412 malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). *Am J Trop Med Hyg.* 2007;77:119–127.
5. Kifude CM, Rajasekariah HG, Sullivan DJ, Jr., Stewart VA, Angov E, Martin SK, Diggs CL, 440 Waitumbi JN. Enzyme -linked immunosorbent assay for detection of Plasmodium 441 falciparum histidine -rich protein 2 in blood, plasma, and serum. *Clin Vaccine Immunol.* 2008;15:1012–1018.
6. Martin SK, Rajasekariah GH, Awinda G, Waitumbi J, Kifude C. Unified parasite lactate 504 dehydrogenase and histidine-rich protein ELISA for quantification of Plasmodium falciparum. *Am J Trop Med Hyg.* 2009;80:516–522.
7. Moody A. Rapid diagnostic tests for malaria parasites. *Clin Microbiol Rev.* 2002;15:66–78.

Warranty

EMD Millipore Corporation warrants its products will meet their applicable published specifications when used in accordance with their applicable instructions for either: a) a period of one year from the date of shipment of the products; or b) a term less than one year, as such term is expressly stated on the product specification or product label. **EMD MILLIPORE MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.** The warranty provided herein and the data, specifications, and descriptions of EMD Millipore products appearing in EMD Millipore's published catalogs and product literature may not be altered except by express written agreement signed by an officer of EMD Millipore. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and if given, should not be relied upon.

In the event of a breach of the foregoing warranty, EMD Millipore's sole obligation shall be to repair or replace, at its option, the applicable product or part thereof, provided the customer notifies EMD Millipore promptly of any such breach. If after exercising reasonable efforts, EMD Millipore is unable to repair or replace the product or part, then EMD Millipore shall refund to the Company all monies paid for such applicable product. **EMD MILLIPORE SHALL NOT BE LIABLE FOR CONSEQUENTIAL, INCIDENTAL, SPECIAL, OR ANY OTHER DAMAGES RESULTING FROM ECONOMIC LOSS OR PROPERTY DAMAGE SUSTAINED BY ANY COMPANY CUSTOMER FROM THE USE OF ITS PRODUCTS.**

© 2018 EMD Millipore Corporation. All rights reserved. No part of these works may be reproduced in any form without permission in writing.

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses.