Malaria P.f./P.v./Pan FIA Rapid Test Device
(Whole Blood)
FI2-MLR-001

A Fluorescence Immunoassay for the qualitative detection for antigens of P. falciparum (P.f.), P. vivax (P.v.) P. ovale (P.o.) and P. malariael (P.m.) (P.f., P.v., Pan.) in human whole blood with the use of the Biopanda Fluorescence Immunoassay Device (BR-FIA-2000).
For professional in vitro diagnostic use only.

INTENDED USE
The Biopanda Malaria P.f./P.v./Pan FIA Rapid Test Device is based on Fluorescence Immunoassay for the *in vitro* qualitative detection of antigens of P. falciparum (P.f.), P. vivax (P.v.), P. ovale (P.o.) and P. malariael (P.m.) (P.f., P.v., Pan.) in human whole blood.

SUMMARY
Malaria is caused by a protozoan which invades human red blood cells.[1] Malaria is one of the world's most prevalent diseases. According to the WHO, the worldwide prevalence of the disease is estimated to be 300-500 million cases and over 1 million deaths each year. Most of these victims are infants and young children. Over half of the world's population lives in malaria zones. Microscopic analysis of appropriately stained thick and thin blood smears has been the standard diagnostic technique for identifying malaria infections for more than a century.[2] The technique is capable of accurate and reliable diagnosis when performed by skilled microscopists using defined protocols. The skill of the microscopist and use of proven and defined procedures, frequently present the greatest obstacles to fully achieving the potential accuracy of microscopic diagnosis. Although there is a logistical burden associated with performing a time-intensive, labor-intensive, and equipment-intensive procedure such as diagnostic microscopy, it is the training required to establish and sustain competent performance of microscopy that poses the greatest difficulty in employing this diagnostic technology.
The Biopanda Malaria P.f./P.v./Pan FIA Rapid Test Device is a test to qualitatively detect the presence of *P. falciparum* - specific HRP-II, *P. vivax* (P.v.) and four kinds of circulating plasmodium falciparum (P.f.), *P. vivax* (P.v.), *P. ovale* (P.o.), and *P. malariae* (P.m.). The test utilizes a combination of antibody coated particles and capture reagents to selectively detect P.f-specific, P. vivax (P.v.) and Pan-malarial antigens (P.f., P.v., P.o. and P.m.) in whole blood.

PRINCIPLE
The Malaria P.f./P.v./Pan FIA Rapid Test Device is based on Fluorescence Immunoassay for the detection of the P.f-specific, P. vivax (P.v.) and Pan-malarial antigens (P.f., P.v., P.o. and P.m.) in human blood specimens. The membrane is pre-coated with anti-HRP-II antibodies, anti-p.v. LDH and anti-pan LDH antibodies. The specimen moves through the strip from sample pad to absorbent pad. If the specimen contains P.f-specific, P. vivax (P.v.) and Pan-malarial antigens (P.f., P.v., P.o. and P.m.), it attaches to the fluorescent microspheres-conjugated antibodies. Then the complex will be captured by the capture antibodies coated on the nitrocellulose membrane (Test line).
The concentration of HRP-II, p.v. LDH and/or pan LDH in the sample correlates with the fluorescence signal intensity captured on the T line, which can be scanned by Biopanda Fluorescence Immunoassay Device. The testing result of of Malaria p.f., p.v. and pan will display on the Biopanda Fluorescence Immunoassay Device screen.

REAGENTS
The test contains anti-Histidine-Rich Protein II (HRP-II) antibody, anti-p.v. LDH antibody and anti-pan LDH antibody conjugated fluorophores and capture reagents coated on the membrane.

PRECAUTIONS
1. For professional *in vitro* diagnostic use only.
2. Do not use after the expiration date indicated on the package. Do not use the test if the foil pouch is damaged. Do not reuse.
3. Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
4. Do not eat, drink or smoke in the area where the specimens and tests are handled. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow standard procedures for proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
5. Do not interchange or mix reagents from different lots.
6. Humidity and temperature can adversely affect results.
7. Used testing materials should be discarded in accordance with local regulations.
8. Read the entire procedure carefully prior to any testing.
9. The Biopanda Malaria P.f./P.v./Pan FIA Rapid Test Device should only be used with the Biopanda Fluorescence Immunoassay Device by approved medical professionals.

STORAGE AND STABILITY
1. The kit should be stored at 4-30°C until the expiry date printed on the sealed pouch.
2. The test must remain in the sealed pouch until use.
3. Do not freeze.
4. Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

KIT COMPONENTS
- 25 x foil wrapped Malaria P.f./P.v./Pan test devices
- 2 x buffer bottles (3 ml)
- 25 x capillary droppers
- 1 x ID card (Malaria P.f./P.v./Pan)
- Package Insert

MATERIALS REQUIRED BUT NOT PROVIDED
- Timer
- Lancets
- Pipette
- Biopanda Fluorescence Immunoassay Device (BR-FIA-2000)

SPECIMEN COLLECTION AND PREPARATION
• Collect the specimen according to standard procedures.
• Do not leave specimens at room temperature for prolonged periods. Whole blood collected by venipuncture should be stored at 2-8 °C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Fingerstick whole blood must be used as soon as possible.

DIRECTIONS FOR USE
Refer to the Biopanda Fluorescence Immunoassay Device Operation Manual for the complete instructions on use of the test. The test should be conducted at room temperature. Cold buffer solution or moisture condensation on the membrane can lead to invalid test results.

Note: There are two test modes for the Biopanda Fluorescence Immunoassay Device: Standard Test mode and Quick Test mode. *Standard Test* mode is a 'set and forget' method where the test will automatically be read after 15 minutes. *Quick* Test mode provides an instant result but the user must monitor the 15 minute test time themselves. It is suitable when running multiple tests concurrently.
Refer to the Biopanda Fluorescence Immunoassay Device Operation Manual for further details.

Allow the test cassette, specimen, buffer and/or controls to reach room temperature (15-30°C) prior to testing.

1. Turn on the Analyser. Then according to users' requirements, select "Standard test" or "Quick test" mode.
2. Take out the ID card and insert it into the Analyser port.
3. Remove the test cassette from the sealed foil pouch and start testing as soon as possible.
4. Place the test on a flat and clean surface.
   Use a capillary dropper: Hold the dropper vertically, draw the specimen to the **fill line** (approximately 10 µL), and transfer the specimen to the specimen well.
   Use a pipette: Transfer **10 µL of specimen** to the specimen well.
5. **After the whole blood specimen is absorbed evenly (about 5-10 seconds)**, add **2 drops of buffer** (about 80 µL) into the sample well. Start the timer at the same time.
6. Results should be read at **15 minutes** with the use of the Biopanda Fluorescence Immunoassay Device.

INTERPRETATION OF RESULTS
The result of tests for Malaria P.f./P.v./Pan is calculated by Fluorescence Immunoassay Analyzer and display the result on the screen. For additional information, please refer to the user manual of Fluorescence Immunoassay Analyzer.
NOTE: The test result of each specimen is given as Pos (+) or Neg (-) with a

Reference Value. This Value is calculated that a measured signal is divided by an appropriate cutoff value.

- Test results of a Value ≥1.0 COI are considered positive.
- Test results of a Value <1.0 COI are considered negative.

QUALITY CONTROL

Each Biopanda Malaria P.f./P.v./Pan FIA Rapid Test Device contains an internal control that satisfies routine quality control requirements. This internal control is performed each time a patient sample is tested. This control indicates that the test device was inserted and read properly by the Biopanda Fluorescence Immunoassay Device. An invalid result from the internal control causes an error message on the Biopanda Fluorescence Immunoassay Device indicating that the test should be repeated. An invalid result from the internal control causes an "N/A" message on the Biopanda Fluorescence Immunoassay Device. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

LIMITATIONS

1. The Malaria P.f./P.v./Pan FIA Rapid Test Device is for professional *in vitro* diagnostic use, and should only be used for the qualitative detection of Malaria p.f., p.v. and pan antigens.
2. The Malaria P.f./P.v./Pan FIA Rapid Test Device will only indicate the presence of Malaria p.f., p.v. and pan antigens in the specimen and should not be used as the sole criterion for the diagnosis of malaria infection.
3. As with all diagnostic tests, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.
4. The results of Malaria P.f./P.v./Pan Tests are based on measuring the levels of Malaria p.f., p.v. and pan antigens in a specimen. It should not be used as the sole criterion for treatment decisions. If the result is positive, other clinical findings and alternative test methods are recommended to reach proper medical treatments.

PERFORMANCE CHARACTERISTICS

SENSITIVITY AND SPECIFICITY

The results show that the specificity and sensitivity of the Malaria P.f./P.v./Pan FIA Rapid Test Device is 99.3% and 98.7%, when compared to results obtained with microscopy.

Method		Microscopy		Total Results
Malaria P.f./P.v./Pan FIA Rapid Test Device	Results	Positive	Negative	
	Positive	77	1	78
	Negative	1	148	149
Total results		78	149	227

Relative Sensitivity: 98.7% (95%CI*: 93.1%~100%)
Relative Specificity: 99.3% (95%CI*: 96.3%~100%)
Accuracy: 99.1% (95%CI*: 96.8%~99.9%) *Confidence Intervals

PRECISION

INTRA-ASSAY

Within-run precision has been determined by using 10 replicates of 6 specimens: P.f. low positive, P.f. middle positive, P.v. low positive, P.v. middle positive, Pan low positive, Pan middle positive. The test results of the specimens were correctly identified >99% of the time.

INTER-ASSAY

Between-run precision has been determined by 10 independent assays on the same 6 specimens: P.f. low positive, P.f. middle positive, P.v. low positive, P.v. middle positive, Pan low positive, Pan middle positive. The test results of the specimens were correctly identified >99% of the time.

CROSS-REACTIVITY

The Malaria P.f./P.v./Pan FIA Rapid Test Device has been tested by HAMA, RF, HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, anti-Syphilis, anti-HIV, anti-HCV, anti-*H. Pylori*, anti-MONO, anti-CMV IgM, anti-Rubella IgM and anti-TOXO IgM positive specimens. The results showed no cross-reactivity.

Interfering Substances

The following potentially interfering substances were added to Malaria low and middle positive specimens.

Acetaminophen: 20 mg/dL	Caffeine: 20 mg/dL
Acetylsalicylic Acid: 20 mg/dL	Gentisic Acid: 20 mg/dL
Ascorbic Acid: 2 g/dL	Albumin: 2 g/dL
Creatin: 200 mg/dL	Bilirubin: 1 g/dL
Oxalic Acid: 60 mg/dL	

None of the substances at the concentration tested interfered in the assay.

REFERENCES

1. Bill MaConell, Malaria Laboratory Diagnosis. January 2001.
2. Cooke AH, Chiodini PL, Doherty T, et al, Comparison of a parasite lactate dehydrogenase-base immunochromatographic antigen detection assay with microscopy for the detection of malaria parasite in human blood samples. Am J Trop Med Hyp, 1999, Feb: 60(2):173-2.

Index of Symbols

	Manufacturer	Σ	Tests per kit	2	Do not reuse test
IVD	*In vitro* diagnostic medical device		Expiration date	REF	Catalogue number
	Store between 4-30°C	LOT	Lot Number		Consult instructions for use
	Do not use if package is damaged				

Thank you for purchasing Biopanda's Malaria P.f./P.v./Pan FIA Rapid Test Device. Please read this manual carefully before operating to ensure proper use.

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