Pf- Xpat Malaria *P.f* (HRP2/pLDH) Test

Product codes	M002-C0001	M002-C0002
Pouch sealed tests	25	40
Single-use inverted cups	25	40
Single-use sterile lancets	25	40
Alcohol swabs	25	40
Assay buffer	Two 5ml bottles	Two 5ml bottles
Instructions for use	1	1

INTENDED USE

The Pf- Xpat Malaria *P.f* (HRP2/pLDH) Test is an immunochromatographic, rapid diagnostic assay intended for the qualitative detection of *Plasmodium falciparum* specific histidine-rich protein-2 (*P.f* HRP2) antigen and *Plasmodium falciparum* specific pLDH (*P.f* pLDH) antigen in human finger stick whole blood and venous whole blood. It is intended for use by only trained users. It is used as an aid for the diagnosis of *P. falciparum* Malaria infection. Results from the Pf-Xpat Malaria *P.f* (HRP2/pLDH) Test should not be used as the sole basis for diagnosis.
For in vitro diagnostic use only. For professional use only.

PRINCIPLE OF THE PROCEDURE

Pf-Xpat Malaria *P.f* (HRP2/pLDH) Test is a membrane-based immunochromatographic assay that uses a nitrocellulose membrane pre-coated with monoclonal antibodies specific to histidine-rich protein-2 (HRP2) of *Plasmodium falciparum* in test line 1 (T1) and monoclonal antibodies specific to plasmodium lactate dehydrogenase (pLDH) of *P. falciparum* in test line 2 (T2). This test utilizes the principles of immunochromatography to detect two targets: *P. falciparum* specific histidine-rich protein-2 (*P.f* HRP2) and *P. falciparum* Specific Lactate dehydrogenase (*P.f* pLDH) in human finger stick whole blood and venous whole blood.
After adding the specimen into the sample well and adding buffer into the buffer well, the specimen-buffer mixture migrates towards the other end of the cassette by capillary action. The antigen in the specimen binds to the antibody-gold colloid to form the antigen-antibody-gold colloid complex. The complex migrates further to capture *P.f* HRP2-specific antibody and (or) *P.f* pLDH-specific antibody pre-coated on the test line T1 or T2, respectively of the nitrocellulose membrane. When the target antigen (*P.f* HRP2 and/or *P.f* pLDH) level is at or above the lower limit of detection (LoD) of the assay (100 parasites/µL), a visible red/pink test line will appear in T1 or T2, or both T1 and T2 indicating a positive result.
Rabbit IgG – gold colloid combines with goat anti-rabbit IgG polyclonal antibody in the control (C) line to form a red reaction line. The control line is used for procedural control indicating that the buffer has been applied successfully and that the active ingredients of the main components on the strip are functional.

WARNINGS

For In-Vitro Diagnostic Use Only

1. Read and understand the instructions for use completely before using this assay. Follow the instructions carefully as not doing so may result in inaccurate test results.
2. **Do not use any other liquid other than the buffer provided during the assay.**
3. Use the test device only with the specified specimen types. Using unauthorized specimen types may lead to inaccurate results.
4. This test assay should be performed at 20%-90% humidity and 15°C- 40°C room temperature. If stored in a refrigerator, ensure that all components return to room temperature before they are put to use.
5. Do not open the sealed pouch until you are ready to conduct the assay. Once opened, use the test cassette with in 1 hour.
6. Do not use the kit contents after the expiry date has been reached.
7. Do not use the test cassette if the pouch is punctured or improperly sealed.
8. Ensure you read the results in a well-lit room to avoid inaccurate reading of the results.
9. Results should be read not earlier than 15 minutes and not later than 30 minutes from the time of buffer addition as doing so will result in erroneous results.
10. Failure to add the sample and the buffer in the correct order as stated in the procedure shall result in erroneous results.
11. Specimens from individuals infected with *Plasmodium falciparum* malaria who are receiving antimalarial drugs may produce false negative test results.
12. Do not mix a buffer and a test cassette from different lots.

PRECAUTIONS

Safety precautions

1. Handle the samples and the materials that are in contact with the sample as potentially infectious.
2. Ensure to put on personal protective equipment like disposable gloves, laboratory coats when handling specimens and performing tests.
3. Avoid splashing and aerosol formation.
4. The process for disposing of the diagnostic device must follow local infectious waste disposal laws or laboratory regulations.
5. Decontaminate and dispose of all specimens, reaction kits and potentially contaminated materials as biohazardous waste.
6. Clean and disinfect all spills using 0.5% bleach or any other appropriate disinfectant.
7. The Buffer contains 2% Triton X-100, which presents no hazard to the user if normal laboratory safety precautions are followed. Wear protective gloves. In case of contact with the skin and/or the eyes, rinse with water immediately. If irritation or signs of toxicity occur, seek medical attention. Follow local regulations or laws to dispose of the Buffer.
8. Keep out of reach of children.

Handling precautions

a. Each test cassette, disposable inverted cup, lancet and alcohol prep pad is for single-use only.
b. Do not use if the desiccant is missing or if the orange-colored particles have turned green. Discard the test device and use a new one.
c. Open only the red cap of the diluent buffer.

KIT CONTENTS

A. Kit Components

Each individually sealed pouch contains one test cassette and one desiccant pouch (for storage purposes only).
There are two configurations of the test kit.

The kit component configurations are provided below:

Components \ Catalogue No.	M002-C0001	M002-C0002
Cassette (pcs)	25	40
Instruction for use (pcs)	1	1
Buffer	2x5mL/bottle	2x5mL/bottle
Inverted cups (pcs)	25	40
Alcohol prep pads (pcs)	25	40
Blood lancet for single use (pcs)	25	40

B. Reactive ingredients of main components

The test strip is coated with gold conjugates (Malaria *P.f* HRP2 monoclonal antibody-gold colloid, Malaria *P.f* pLDH monoclonal antibody-gold colloid, rabbit IgG-gold colloid), test line 1 (Malaria HRP2 monoclonal antibody), test line 2 (Malaria *P.f* pLDH monoclonal antibody) and control line (goat anti-rabbit IgG polyclonal antibody).

MATERIALS REQUIRED BUT NOT PROVIDED

1. Timer
2. Protective gloves
3. Specimen and test waste container
4. Cotton swab/ball

Note: If whole blood is collected by venipuncture, venipuncture blood collection materials are required in addition to a precision pipette and pipette tips.

STORAGE AND STABILITY

1. Store the kit at 1°C- 40°C. The shelf life of the test kit is 24 months.
2. Use the test cassette within 1 hour after opening. For buffer, store at 1°C -40°C for no more than 8 weeks after opening.
3. Use the kit at 20% - 90% humidity and 15°C - 40°C, on a clean and flat surface.
4. Recap and store the buffer in the original container after use.
5. Keep away from sunlight moisture, and heat.
6. Do not freeze.

SPECIMEN COLLECTION AND STORAGE

Finger stick whole blood:

1. Wash your hands and put on protective gloves. Select the middle or ring finger of the patient and avoid calloused areas of the finger. Clean the fingertip to be lanced with an alcohol prep pad.
2. Prick the lateral side of the fingertip with a lancet. Gently squeeze the end of the fingertip and wipe off the first drop of blood with a sterile cotton swab/ball. Squeeze the fingertip again and allow a new drop of blood to form.
3. Draw 5 microliters (5µL) of blood using an inverted cup. Test immediately after the whole blood specimen is collected by finger stick.

Venous whole blood:

1. Draw venous whole blood using standard phlebotomy procedures into a blood collection tube with anticoagulant (EDTA, sodium citrate, or heparin sodium).
2. If immediate testing is not possible, the specimen may be stored at 2°C–8°C for up to 72 hours before testing.
3. The specimens should be stored at -70°C or below for longer storage.

Note: Prior to testing, bring specimens to room temperature slowly and mix well gently once thawed. Do not freeze and thaw the specimens repeatedly. In case repeated freezing and thawing of specimens is required, no more than 5 cycles should be allowed.

TEST PROCEDURE

Bring all specimens and kit components to room temperature (15°C–40°C) before testing. When ready to test, remove the test cassette from the foil pouch by tearing the notch and place it on a flat, dry surface. Label the device with the specimen ID number.

1. Test Procedure for finger stick whole blood

A. Wipe the finger to be lanced with an alcohol prep pad. Select the middle or ring finger for sampling and avoid calloused areas of the finger.

1

B. Prick the lateral side of either the ring finger or middle finger. Wipe off the first drop of blood with a cotton swab/ball. Squeeze the fingertip again and allow a new drop of blood to form.

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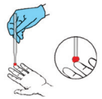
C. Dip the circular end of the inverted cup into the blood specimen (5µL).

4



D. Place the circular end of the inverted cup into the sample well.

5

E. **Open only the red cap of the diluent buffer**, then add 4 drops (about 100µL) of buffer into the buffer well vertically, and start timing.

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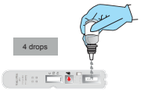
7



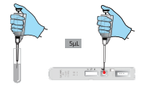
8

15-30 mins

2. Test procedure for venous whole blood

A. Gently mix the venous blood specimen by inverting the blood collection tube to make it homogenous. Take 5µL venous whole blood specimen using a pipette and drop it into the center of the sample well vertically.

1



B. **Open only the red cap of the diluent buffer,** then add 4 drops (about 100µL) of buffer into the buffer well vertically, and start timing.

2

3

4

15-30 mins

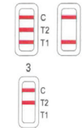
3. Interpretation

A. Read the results after 15 minutes and not later than 30 minutes.
B. To avoid confusion, discard the test device after reading the result.

QUALITY CONTROL

1. Internal control: this kit contains a built-in control feature, the control region (C). A colored line develops in the control region (C) after addition of the specimen and buffer. If a colored line does not develop in the C region, the test is invalid. Review the procedure and repeat the test with a new device.
2. External control: the test kit does not include external control.

INTERPRETATION OF RESULTS

Picture	Lines that you see	Reading and interpretation of Results
	No red line at 'C' (control)	**Result: Invalid.** Action: Take a new cassette and repeat the test. if the specimen is lipaemic or icteric, collect a fresh specimen.
	Red line at 'C' and No other line	**Result: Negative** Action: No
	Red line at 'C' and at 'TI' and/or 'T2'	**Result: Positive.** 1. Line at 'C', 'TI' and 'T2' indicates positive for *P.f* HRP2 and *P.f* pLDH. 2. Line at 'C' and 'TI' indicates positive for *P.f* HRP2. 3. Line at 'C' and 'T2' indicates positive for *P.f* pLDH. **Note:** Line intensity may vary from faint to strong intensity. Consider a faint test line as a positive result.

LIMITATIONS OF PROCEDURE

1. The kit is only a qualitative assay designed to detect *P. falciparum* specific histidine rich protein-2 (*P.f* HRP2) and *P. falciparum* specific pLDH (*P.f* pLDH) antigens in human whole blood only.
2. Strictly operate according to the instructions for use when testing. Incorrect operation will result in false results.
3. Use EDTA, heparin sodium, and sodium citrate as anticoagulants. Do not use other anticoagulants.
4. False negative results can occur in the following conditions:
   - Very low antigen concentration/parasite densities, for instance <100 parasites /μl. Note that most clinical cases have higher parasite densities.
   - Very high parasite densities (very exceptional, prozone or high-hook effect) for HRP2 antigen. Unlike the *P.f* HRP2 antigen, *P.f* pLDH is not susceptible to the prozone effect.
   - Deletions in the HRP2 gene result in no production of the HRP2 antigen, which is of particular relevance for mRDTs that detect this antigen. 25.7%- 41.0%.of *P. falcipurum* species in the Peruvian amazon lack the *P. falcipurum* HRP2 gene and these deletions have since been reported in various malaria-endemic regions worldwide. In that case, *P.f* pLDH could possibly identify the *P. falcipurum* infections.
   - High fraction of interstitial fluid due to “milking” of fingertip.
5. The presence of the control line only means that migration of added liquid occurred. It does not guarantee that:
   - The correct specimen has been used.
   - The specimen has been applied correctly.
   - The specimen and kit have been correctly stored.
   - The test procedure has been followed correctly.
6. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test but instead should be determined by a health care provider in conjunction with clinical findings and the results from other laboratory tests and evaluations. Results from Pf-Xpat Malaria *P.f* (HRP2/pLDH) Test should not be used as the sole basis for diagnosis.
7. The product can give a false positive *P.f* result even after the patient was treated for malaria several weeks before testing because of persistence of *P.f* HRP2 antigen after treatment. However, *P.f* pLDH clears 2 days after treatment and is a more reliable biomarker for results interpretation after treatment. However, the test cannot be used to monitor treatment response to antimalarials.

PERFORMANCE CHARACTERISTICS

Clinical Performance Studies

1. Internal Evaluation

Reference method	Pf-Xpat Malaria *P.f* (HRP2/pLDH) Test			
	Positive	Negative	Total	Test Result (95% CI)
P.f Positive	102	0	102	Sensitivity:100.00% (96.37-100.00%)
P.f Negative	1	625	626	Specificity:99.84% (99.91-99.97%)

Specimen type (s) used in the performance evaluation studies: venous whole blood and finger stick whole blood.

2. External Evaluation

Diagnostic Sensitivity and Specificity

Reference method	Pf-Xpat Malaria *P.f* (HRP2/pLDH) Test			
	Positive	Negative	Total	Test Result (95% CI)
P.f Positive	183	0	183	Sensitivity:100.00% (97.94-100.00%)
P.f Negative	3	354	357	Specificity:99.16% (97.56-99.71%)
P.f Positive	175	0	175	Sensitivity:100.00% (97.85-100.00%)

P.f Negative	0	364	364	Specificity:100.00% (98.96-100.00%)
P.f Positive	133	0	133	Sensitivity:100.00% (97.19-100.00%)
P.f Negative	1	375	376	Specificity:99.73% (98.51-99.95%)

Specimen type used for Reference method: venous whole blood; Specimen type used in the study: finger stick whole blood.

Comparison among different specimen types

Pf-Xpat Malaria *P.f* (HRP2/pLDH) Test		Reference method		Test Result (95% CI)
		Venous whole blood		
		Positive	Negative	
Venous whole blood	Positive	26	0	Sensitivity: 100% (87.13%-100.00%)
	Negative	0	30	Specificity: 100% (88.65%-100.00%)
Finger stick whole blood	Positive	26	0	Sensitivity: 100% (87.13%-100.00%)
	Negative	0	30	Specificity: 100% (88.65%-100.00%)

3. Analytical performance Studies

1. The repeatability and reproducibility of Pf-Xpat Malaria *P.f* (HRP2/pLDH) Test are of good performance demonstrated by in-house panel according to CLSI EP-05 (A3). 100% reproducibility was observed in studies within run, between run, between lots, and between sites and operators.
2. The following potential interfering substances have no impact on the Pf-Xpat Malaria *P.f* (HRP2/pLDH) Test.

No.	Type	Interfering substances
1	Endogenous	HAMA
2		Recipient of multiple blood transfusion
3		Anti-dsDNA antibody
4		Anti-cardiolipin antibody IgG
5		Anti-cardiolipin antibody IgM
6		Rheumatoid factors
7		IgG
8		IgM
9		Albumin
10		total bilirubin
11		Triglyceride
12		Human Chorionic Gonadotropin
13		Cholesterol
14		Low density lipoprotein
15		Haemoglobin

16	Exogenous	Direct bilirubin
17		Chloroquine Phosphate
18		Artemisinin
19		Amoxicillin
20		Cefalexin
21		Doxycycline Hyclate
22		Erythromycin
23		Ibuprofen
24		Piperaquine
25		Mebendazole
26		Tribendimidine
27		Entecavir
28		Rifampicin
29		Aspirin
30		Paracetamol
31		Ethanol
32		Caffeine

3. The following potential cross-reacting pathogens have no impact on Pf-Xpat Malaria *P.f* (HRP2/pLDH) Test.

No.	Type	Cross-reacting substances
1	Antigen	*Brucella spp.*
2		HBsAg
3		Dengue Virus Type 1
4		HIV-1
5		HCV
6		Syphilis
7		*Leishmania spp.*
8		*Leptospira spp.*
9		*M. tuberculosis*
10		*Schistosoma spp.*
11		*Toxoplasma gondii*
12		*Plasmodium vivax*
13		HAV
14		Measles
15		Influenza
16		Tick borne encephalitis

17	Antibody	Rubella-IgG
18		Dengue Virus IgG
19		Cytomegalovirus-IgG

The Limit of Detection of the Malaria *P.f* (HRP2/pLDH) Test for *P.f* HRP2 (T1 line) and *P.f* pLDH (T2 line) is 15.63 IU/mL (NIBSC 16/376 1:128) and 31.25 IU/mL (NIBSC 16/376 1:64), respectively.

REFERENCES

1. WHO. TSS-3 Malaria rapid diagnostic tests, Geneva: World Health Organization; 2017.
2. WHO. TGS-5 Designing instructions for use for in vitro diagnostic medical devices, Geneva: World Health Organization; 2017.
3. WHO. Malaria rapid diagnostic test performance. Results of WHO product testing of malaria RDTs: round 7 (2015-2016), Geneva: World Health Organization; 2017.
4. WHO. Global technical strategy for malaria 2016-2030, Geneva: World Health Organization; 2015.
5. Wondfo One Step Malaria HRP2/pLDH (*P.f*/Pan), Product Instructions for Use, Version No: Rel.: 2024/9/5

SYMBOL KEY

REF	Catalogue number	LOT	Batch code
	Manufacturer		Use-by date
	Date of manufacture		Keep Dry
	Temperature limit		Caution
	Consult *instructions for use*		*Do not re-use*
	Keep away from sunlight		
Σ	Contains sufficient for <*n*> tests		
IVD	In vitro diagnostic *medical device*		
	Do not use if package is damaged and consult *instructions for use*		

Microhaem Scientifics and Medical Supplies Ltd (MHS)
Plot 16, A-C, Martyrs' Way, Ntinda, Kampala, Uganda
Tel: +256393246401
Website: www.microhaem.co.ug

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