Malaria Plasmodium Falciparum (P.f.)/Pan Rapid Test Kit
Instructions For Use

PRODUCT NAME

FRENOVO Malaria Plasmodium Falciparum (P.f.)/Pan Rapid Test Kit

PACKAGE SPECIFICATION

20 tests/kit

INTENDED USE

FRENOVO Malaria Plasmodium Falciparum (P.f.)/Pan Rapid Test is a rapid chromatographic immunoassay for the qualitative detection of four kinds of circulating plasmodium falciparum (P.f.), P. vivax (P.v.), P. ovale (P.o.), and P. malariae (P.m.) in whole blood.

SUMMARY AND PRINCIPLES OF THE PROCEDURE

Malaria is caused by a protozoan which invades human red blood cells. 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, young children. Over half of the world's population lives in malarious areas. 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.

FRENOVO Malaria Plasmodium Falciparum (P.f.)/Pan Rapid Test is a rapid test to qualitatively detect the presence of P. falciparum - specific HRP-II 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 colloid gold conjugate to selectively detect P.f-specific and Pan-malarial antigens (P.f., P.v., P.o. and P.m.) in whole blood.

FRENOVO Malaria Plasmodium Falciparum (P.f.)/Pan Rapid Test is a qualitative, membrane based immunoassay for the detection of P.f., P.v., P.o. and P.m. antigens in whole blood. The membrane is pre-coated with anti-HRP-II antibodies and anti-Aldolase antibodies. During testing, the whole blood specimen reacts with the dye conjugate, which has been pre-coated on the test cassette. The mixture then migrates upward on the membrane by capillary action, reacts with anti-Histidine-Rich Protein II (HRP-II) antibodies on the membrane on P.f. Test Line region and with anti-Aldolase antibodies on the membrane on Pan Line region. If the specimen contains HRP-II or Plasmodium-specific Aldolase or both, a colored line will appear in P.f. line region or Pan line region or two colored lines will appear in P.f. line region and Pan line region. The absence of the colored lines in P.f. line region or Pan line region indicates that the specimen does not contain HRP-II and/or Plasmodium-specific Aldolase. To serve as a procedure control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred

MATERIALS PROVIDED

Each kit contains:

1. test cassettes: 20 pieces test cassettes individually pouched.
2. Wash Buffer Solution: 3.0 ml in dropper bottle.
3. Droppers: 20 pieces droppers of 10 ul.
4. Package insert: 1 piece attached.

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer or stopwatch.
- Specimen collection containers
- Disposable gloves and/or protective clothing
- Micro-pipette

WARNINGS

1. Read the package insert completely before using the product. The instructions must be followed carefully as not doing so may result in inaccurate results.
2. The kits for diagnostic use only.
3. Perform test at room temperature.

PRECAUTIONS

1. FRENOVO Malaria Plasmodium Falciparum (P.f.)/Pan Rapid Test Kit is for professional use only.
2. The package insert instructions must be followed to ensure optimum test performance.
3. The kit is intended for *in vitro* diagnostic use.
4. As with all screening assays, any results should be considered presumptive until confirmatory assays have been performed according to local practice or WHO guidelines.

Safety Precautions

1. Standard precautions for handling infectious agents should be observed when using this kit.
2. Wear protective clothing such as lab coat, safety glasses and disposable gloves when handling specimens and assay reagents.
3. Wash hands thoroughly after use.
4. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Bio safety Precautions

Appropriate bio safety practices should be used when handling specimens and reagents. These precautions include, but are not limited to the following:

1. Do not smoke,eat,drink,apply cosmetics or handle contact lenses in areas in which specimens are handled.
2. Dispose of all specimens, used devices and tubes as though they are capable of transmitting infection. The preferred methods of disposal are by autoclave at 121°C for a minimum of 60 minutes or by incineration. Disposable materials may be incinerated. Liquid waste may be mixed with appropriate chemical disinfectants. A solution of 10% bleach is recommended. Allow 60 minutes for effective decontamination. NOTE: Do not autoclave solutions containing bleach.
3. When disposing of solution, avoid contact with acid to prevent liberation of a toxic gas.
4. All spills should be wiped thoroughly using a suitable disinfectant such as a sodium hypochlorite solution.
5. Use a separate dropper and device for each specimen tested.

Handling Precautions

1. Do not use if the kit safety seal is absent, damaged or broken.
2. Do not use any device if the pouches have been perforated.
3. Each device is for single use only.
4. Do not mix wash buffer solution/test cassettes from different kit lots.
5. Do not use the kit past the expiration date (this date is printed on the kit box).
6. Adequate lighting is required to read the test results.
7. The result should be read immediately after the end of the 10 minutes incubation time following the addition of specimen and wash buffer solution. Do not read results beyond 15 minutes.

STORAGE INSTRUCTIONS

1. The kit should be stored between 2-30°C and the shelf life is 24 months.
2. The kit components are stable until the expiration date printed on the outer label, when stored as directed. The kit expiry date is determined by whichever of the components has the

shortest expiry date. The kit expiry date is not impacted once the wash buffer solution has been opened. Do not use kit components beyond overall kit expiry date.

3. If stored refrigerated, ensure that the pouched device is brought to room temperature before opening.
4. Do not freeze the kit.

SAMPLE COLLECTION AND PREPARATION

1. Applicable samples： Whole Blood
2. Testing should be performed immediately after the specimens have been collected as soon as possible . Do not leave the specimens at room temperature for prolonged periods.
3. Whole blood collected by venipuncture should be stored at 2~8℃ if the test is to be run within 2 days of collection.
4. Do not freeze whole blood specimens.
5. Bring specimens to room temperature prior to testing.
6. If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiological agents.
7. EDTA K2, Heparin sodium, Citrate sodium and Potassium Oxalate can be used as the anticoagulant for collecting the specimen.

QUALITY CONTROL

An internal procedural control is included in the test. a colored line appearing in the control line region (C) is an internal valid procedural control, it confirming adequate membrane wicking. Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

TEST PROCEDURE

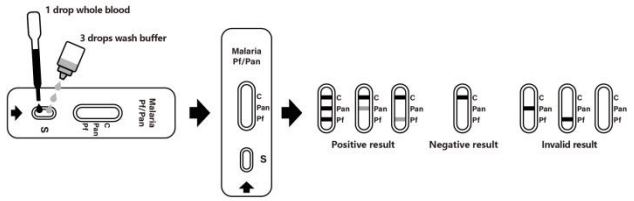
Allow the test cassette, specimen, buffer solution to equilibrate to room temperature (15-30°C) prior to testing.

1. Remove the test cassette from the sealed pouch and use it within one hour. Place the test cassette on a clean and level surface.

2. To use a dropper: Hold the dropper vertically, draw the specimen and transfer the specimen to the sample well of the test cassette (one drop/approximately 10ul), then squeeze the wash buffer solution bottle, add three drops wash buffer solution inside (approximately 90ul) to the sample well and start the timer. Avoid trapping air bubbles in the sample well.

   To use a micro-pipette: Pipette and dispense 10ul of specimen to the sample well of the test cassette, then squeeze the wash buffer solution bottle, add three drops wash buffer solution inside (approximately 90ul) to the sample well and start the timer.

3. Wait for the colored line(s)to appear. The test result should be read at 10 minutes. Do not interpret the result after 15 minutes.



INTERPRETATION OF RESULTS

Negative result: if there is only a quality control line C, the detection line is colorless, indicating that the Malaria antigen has not been detected and the result is negative.

Positive result: if both the quality control line C and the detection lines appear, the Malaria antigen has been detected and the result is positive.

P. falciparum or mixed malaria infection : one line appears in the control region, one line appears in Pan line region and one line appears in P.f. line region.

P. falciparum infection: one line appears in the control region, and one line appears in P.f .line region.

Non-falciparum Plasmodium species infection : one line appears in the control region and one line appears in Pan line region.

Invalid result: Control line fails to appear. Insufficient buffer volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the procedure with a new test cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

NOTE: The intensity of the color in the test line region(s) will vary depending on the concentration of Malaria antigen in the specimen.

LIMITATIONS

1. FRENOVO Malaria Plasmodium Falciparum (P.f.)/Pan Rapid Test is for in vitro diagnostic use only. This test should be used for the detection of P.f., P.v., P.o., P.m. antigens in whole blood specimens only. Neither the quantitative value nor the rate of increase in P.f., P.v., P.o., and P.m. concentration can be determined by this qualitative test.
2. FRENOVO Malaria Plasmodium Falciparum (P.f.)/Pan Rapid Test will only indicate the presence of antigens of Plasmodium sp. (P.f., P.v., P.o., P.m.) in the specimen and should not be used as the sole criterion for the diagnosis of malaria infection.
3. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
4. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of malaria infection.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

Clinical study was performed to compare the results obtained by The kit and PCR. The results indicated that The kit has a high sensitivity and specificity as summarized below:

P.f. clinical study		PCR		
FRENOVO Malaria Pf/Pan Rapid Test	Results	Positive	Negative	Total Results
	Positive	54	8	62
	Negative	1	318	319
Total Results		55	326	381

P.f. Clinical Study Summary Results:

Clinical sensitivity =98.18% (95%CI*90.28%～99.75%)

Clinical specificity = 97.55% (95%CI*95.22%～98.93%)

Accuracy=97.64% (95%CI*95.56%～98.91%)

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P.v. clinical study		PCR		
FRENOVO Malaria Pf/Pan Rapid Test	Results	Positive	Negative	Total Results
	Positive	85	8	93
	Negative	0	318	318
Total Results		85	326	411

P.v. Clinical Study Summary Results:

Clinical sensitivity >99.00% (95%CI*96.54%～100.0%)

Clinical specificity = 97.55% (95%CI*95.22%～98.93%)

Accuracy=98.05% (95%CI*96.20%～99.16%)

Interference Substances

The following potential interfering substances were added to Malaria negative and positive specimens. None of the substances at the concentration tested interfered in the assay.

Substance	Tested Concentration
Hemoglobin	6 mg/ml
Bilirubin	0.4 mg/ml
Triglycerides	15 mg/ml
Cholesterol	4 mg/ml
Human Anti-mouse Antibody (HAMA)	100 IU /ml
Rheumatoid Factor	1500 IU/ml
Antinuclear Antibody (ANA)	100 IU/mL
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	1g/dL
Oxalic Acid	60 mg/dL

Minimum Detection Level

The minimum detection limit for FRENOVO Malaria Plasmodium Falciparum (P.f.)/Pan Rapid Test is 200 Parasites/ul for P.f. and 1500 Parasites/ul for P.v..

Cross Reaction

FRENOVO Malaria Plasmodium Falciparum (P.f.)/Pan Rapid Test has been tested by HAMA, RF, HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, Syphilis, HIV, HCV, H. Pylori, MONO, CMV, Rubella, HSV and TOXO positive specimens. The results showed no cross-reactivity.

INDEX OF SYBOML

	In vitro diagnostic medical device		single-use,Please don't reuse it
	Use-by date		Consult instructions for use
	Cautions		Manufacturer
	Temperature limit		Batch code
	Date of manufacture		Keep Dry
	Avoid overexposure to the sun		Don't use the product when the package is damaged
	CE mark		Biological risks

Hangzhou FRENOVO Biotech Co., Ltd.
Address: Room 401,Building 36,No.488-1,Donghu North Road, Donghu Street, Yuhang District, Hangzhou City, Zhejiang Province, China.
Tel: 86-0571-89170657
Email: business@frenovo.com

EC REP
Lotus NL B.V.
Address: Koningin Julianaplein 10, 1e Verd, 2595AA, The Hague, Netherlands.
Email: peter@lotusnl.com

INSTRUCTION APPROVAL AND REVISION DATE

Revision Date:
Date of Issue: