GENESIS

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EZER™ Malaria Pf/Pan Antigen Rapid Test Device

INTENDED USE

The *EZER*™ Malaria Pf/Pan Antigen Rapid Test Device is a lateral flow chromatographic immunoassay for the qualitative detection of four kinds of circulating plasmodium (P. falciparum, P. vivax, P. ovale, and P. malariae) in human whole blood.

It is intended to be used as a screening test and aid in the diagnosis of infection with plasmodium. Any reactive specimen with the Malaria Pf/Pan Antigen Rapid Test Device must be confirmed with alternative testing method(s) and clinical findings. It is not suitable for general population screening.

BACKGROUND

Malaria is an acute mosquito-borne infectious disease caused by the infection of plasmodium, which invades human red blood cells and seriously endangers the health of human beings. It is characterized by fever with chills, anemia and is caused by Plasmodium parasite that is transmitted from one human being to another by the bite of infected Anopheles mosquitoes.

There are four kinds of parasite that can infect humans: P. falciparum, P. vivax, P. ovale, and P. malariae. Of these, P. falciparum causes more severe disease than the other plasmodial species and accounts for most malaria deaths. P. falciparum and P. vivax are the most common pathogens. The disease exists in more than 90 countries worldwide, and it is estimated that there are over 500 million clinical cases and 2.7 million malaria-caused deaths per year.

The *EZER*™ Malaria Pf/Pan Antigen Rapid Test Device is developed to simultaneously detect and differentiate infection with P. falciparum and the other Plasmodium species.

PRINCIPLE

The *EZER*™ Malaria Pf/Pan Antigen Rapid Test Device is a qualitative lateral flow immunochromatographic assay for the detection of Histidine Rich Protein II (HRP-II) in P. falciparum, and lactate dehydrogenase (pLDH) in all plasmodium.

The test uses Anti-HRP-II antibody 1 (test line T1), Anti-pLDH antibody 1 (test line T2) and goat anti-mouse IgG (control line C) immobilized on a nitrocellulose strip.

During testing, the specimen reacts with Anti-pLDH antibody 2-coated particles and Anti-HRP-II antibody 2-coated particles in the test. The mixture then migrates upward on the membrane chromatographically by capillary action and reacts with Anti-HRP-II antibody 1 in T1 test line region and Anti-pLDH antibody 1 in T2 test line region. If the specimen contains pLDH, a colored line will appear in T2. If the specimen contains HRP-II, a colored line will appear in T1 test line region as a result. Therefore, if the specimen does not contain pLDH or HRP-II, no colored line will appear in the test line region, indicating a negative result. To serve as a procedural control, a colored line will always appear in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

CONTENTS

EZER™ Malaria Pf/Pan test devices (20 pcs), Sample buffer (1 vial), Plastic dropper (20 pcs), Package Insert (1 pc)

WARNINGS AND PRECAUTIONS

1. For *in vitro* Diagnostic Use.
2. Do not use Device components beyond the expiration date.
3. Pathogenic microorganisms may be present in clinical specimens. All specimen and the related contaminated items need to be handled, stored and disposed following “Standard Precautions” and institutional guidelines.
4. Wear protective clothing such as laboratory coats, masks, disposable gloves and eye protection when specimens are handled.
5. Ensure foil pouch containing test device is not damaged before opening for use.
6. The test plate should be used immediately after opening the packaging. When it absorbs moisture, the quality deteriorates and an accurate result cannot be obtained.
7. Please do not touch the sample drop and the judgment part of the test board directly by hand.
8. Do not reuse the device.
9. Use a clean, sterile pipette tip or dropper for each sample.
10. Please ensure that appropriate samples are used for testing. Too much or too little sample size may lead to deviation of results.
11. If the test is invalid, one should consider the possible improper handling, inaccurate operation procedure, or device quality. Repeat the test with a new device ensuring that the test procedure has been followed accurately.
12. Read the result at 10 mins, or up to 20 mins. Do not read the result longer than 20 mins, because the result might be altered if the device is dry out.
13. All samples and used accessories should be treated as infectious and discarded according to local

regulations.

STORAGE CONDITIONS

Test devices must be stored at 2~30°C. **DO NOT FREEZE.** Devices must be at ambient room temperature at time of testing.

SAMPLE COLLECTION AND PREPARATION

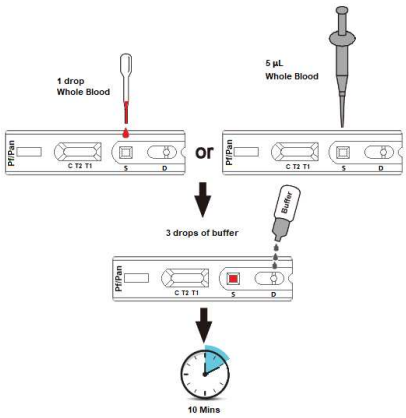
- Applicable samples：Whole Blood.
- Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods. Whole blood collected by venipuncture should be stored at 2~8°C if the test is to be run within 2 days of collection.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

PROCEDURE

Allow the *EZER*™ Malaria Pf/Pan Antigen Rapid Test Device and collected samples to equilibrate to room temperature (15~30°C) prior to testing. Refer to testing procedures printed inside the Device.

1. Bring the specimen and test components to room temperature if refrigerated. Mix the specimen well prior to assay once thawed.
2. Open the foil pouch and take out the reagent plate. Once opened, test immediately. Write down the patient's name or number on the reagent plate. (One patient per reagent plate)
3. **For Whole Blood Specimen**：
   - To use a dropper: Hold the dropper vertically, draw the specimen and transfer 1 full drop (approx. 5μL) of specimen to the sample well. Then add 3 drops of buffer (approximately 90μL) and start the timer.
   - To use a pipette: To transfer 5μL of whole blood to the specimen well, then add 3 drops of buffer (approximately 90μL), and start the timer
     See illustration below.

Whole Blood Specimen：



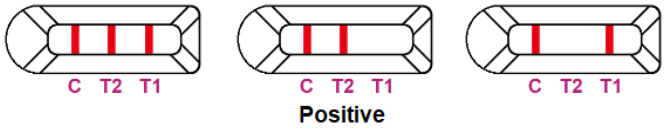
4. **Read the results after 10 minutes. If more than 20 minutes, please retest with another test device.**

Note: It is suggested not to use the buffer, beyond 6 months after opening the vial.

INTERPRETATION OF RESULTS

Allow the samples to react according to the procedure and read the red purple lines that appear in the reading area.

Positive:



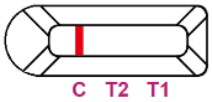
The colored line in the control line region (C) appears and a colored line appears in test line region (T1) or test line region (T2).

P. falciparum or mixed malaria infection:
The colored line in the control line region (C) appears and colored lines appear in test line regions T1 and T2. The color intensities of the lines do not have to match. The result is positive for P. falciparum. In some cases, the appearance of both the T1 and T2 Lines may indicate a mixed infection of P.f. with another species.

Non-falciparum Plasmodium species infection:
The colored line in the control line region (C) appears and a colored line appears in test line region T2. The result is positive for P. vivax, P. ovale or P. malariae. The appearance may indicate a mixed infection with two or more of P. vivax, P. ovale and P. malariae in some cases.

P. falciparum infection:
The colored line in the control line region (C) appears and a colored line appears in test line region T1. The result is positive for P. falciparum.
※NOTE: The intensity of the color in the test line region (T1 or T2) will vary depending on the concentration of P. falciparum, P. vivax, P. ovale or P. malariae in the specimen. Therefore, any shade of color in the test line regions should be considered positive.

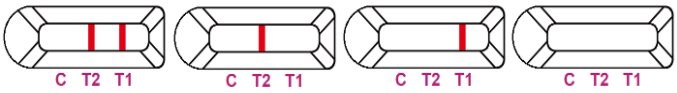
Negative Result:



Negative

The colored line in the control line region (C) appears. No line appears in test line regions (T1 or T2).

Invalid:



Invalid

Control band 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 device. If the problem persists, discontinue using the test Device immediately and contact your local distributor.

PRECAUTIONS FOR ASSESSMENT

1. Assessment must be conducted exactly 10 minutes after starting the reaction. Given the nature of the measurement (immunochromatography), the reaction and color development may slightly continue and progress even after 10 minutes.
2. The color tone of the line may vary depending on the color tone and specimen properties. However, the test result is valid as long as a red line is present. Occasionally, broken lines may appear, but the test result is valid as long as a red line is present.
3. If the specimen is dark-colored, it may stain the membrane and affect the assessment.
4. A highly viscous specimen may affect sample migration and/or the reaction, resulting in weak coloration, delayed or no formation of the line, or a nonspecific reaction because of specimen retention.

QUALITY CONTROL

Internal procedural controls are included in the test. A colored line appearing in the control region (C) is an internal procedural control. It confirms sufficient specimen volume and correct procedural technique. Control standards are not supplied with this Device; 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.

PERFORMANCE CHARACTERISTICS

Sensitivity and specificity

The *EZER*™ Malaria Pf/Pan Antigen Rapid Test Device was compared with microscopy on clinical samples; the results show that *EZER*™ Malaria Pf/Pan Antigen Rapid Test Device has a high sensitivity and specificity.

Result for P.falciparum：

		Microscopy		
		+	-	Total
The *EZER*™ Malaria Pf/Pan	+	47	0	47
	-	2	197	199
	Total	49	197	246

Relative Sensitivity：95.92% (95%CI*: 86.02%～99.50%)
Relative Specificity：＞99.99% (95%CI*: 98.14%～100.00%)
Accuracy：99.19% (95%CI*: 97.09%～99.90%)
*Confidence Interval

Result for Plasmodium non-falciparum species:

		Microscopy		
		+	-	Total
The *EZER*™ Malaria Pf/Pan	+	41	0	41
	-	1	197	198
	Total	42	197	239

Relative Sensitivity：97.62% (95%CI*: 87.43%～99.94%)
Relative Specificity：＞99.99% (95%CI*: 98.14%～100.00%)
Accuracy：99.58% (95%CI*: 97.69%～99.99%)
*Confidence Interval

Minimum Detection Level

Type	Parasites/μL
P. falciparum	100
Plasmodium non-falciparum species	100

Cross-reactivity evaluation

The *EZER*™ Malaria Pf/Pan Antigen Rapid Test Device has been tested for anti-influenza A virus, anti-influenza B virus, anti-RSV, anti-Adenovirus, HBsAg, anti-Syphilis, anti-H. Pylori, anti-HIV and anti-HCV positive specimens. The results showed no cross-reactivity.

Interfering Substances

The following compounds have been tested using the *EZER*™ Malaria Pf/Pan Antigen Rapid Test Device and no interference was observed.
Triglyceride: 50 mg/dL
Ascorbic Acid: 20mg/dL
Hemoglobin: 1000mg/dL
Bilirubin: 60mg/dL
Total cholesterol: 6mmol/L

LIMITATIONS OF THE PROCEDURE

1. The *EZER*™ Malaria Pf/Pan Antigen Rapid Test Device is for *in vitro* diagnostic use only. This test should be used for detection of four kinds of circulating plasmodium in whole blood.
2. Neither the quantitative value nor the rate of increase in the concentration of P. falciparum, P. vivax, P. ovale, and P. malariae antigens can be determined by this qualitative test.
3. The *EZER*™ Malaria Pf/Pan Antigen Rapid Test Device will only indicate the presence of antigens of plasmodium in the specimen and should not be used as the sole criterion for the diagnosis of malaria infection.
4. A negative result can occur if the quantity of plasmodium present in the specimen is below the detection limits of the assay.
5. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
6. The results obtained with this test should be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

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Index of Symbols

	Attention, see instructions for use		Tests per kit	EC REP	Authorized Representative
IVD	For *in vitro* diagnostic use only		Use by		Do not reuse
	Store between 2~30°C	LOT	Lot Number	REF	Catalog #

EC REP **Lotus NL B.V.**
Koningin Julianaplein 10, le Verd, 2595AA,
The Hague, Netherlands.

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