Interpretation of Assay Result

1.NEGATIVE: If only the C line is present, the absence of any burgundy color in both test lines (Pan and Pf) indicates that the plasmodium antigens are not detected. The result is negative or non-reactive.

2. POSITIVE RESUL T:
2.1 In addition to the presence of the C line, if only the Pan line develops, the test indicates the presence of pLDH antigen. The result is Pf negative or non-reactive, and positive or reactive for any of the other three Plasmodium species (Pv, Pm and Po) (Subject Limitations of Test 6).

2.2 In addition to the presence of the C line, if both Pan & Pf line develops, the test indicates the presence of pHRP-II antigen which indicates Pf infection(Pf will be also dictated by coated pLDH antibody) . The result is Pf positive or reactive. or this result indicate presence of both pHRP2 & pLDH antigen, which means the specimen contain both Pf & Pan Infection

Samples with positive results should be confirmed with alternative testing method(s) and clinical findings before a diagnosis is made

3. INVALID:
If no C line develops, the assay is invalid regardless of any burgundy color in the test lines as indicated below. Repeat the assay with a new device.

Performance Characteristics

1. Clinical Performance

1. 1 Performance for Pf Ag Test
A total of 361 blood specimens were collected from a malaria endemic area (throughout India) and tested by the CADY Malaria Pf/PAN Ag Rapid Test in comparison with thick blood smear test. Comparison for all the specimens is shown in the following table:

Smear Test	Positive	Negative	Total
Positive	60	1	61
Negative	0	300	300
Total	60	301	361

Relative Sensitivity:99.65% Relative specificity 100%,overall Agreement:99:83%

1. 2 Performance for Pan Ag T est
A total of 591 blood specimens were collected from a malaria endemic area (throughout India) and tested by the CADY Malaria Pf/PAN Ag Rapid Test in comparison with thick blood smear test. Comparison for all the specimens is shown in the following table.

Smear Test	Positive	Negative	Total
Positive	290	1	291
Negative	0	300	300
Total	290	301	591

Relative Sensitivity:99.65% Relative specificity 100%,overall Agreement:99:83%

2. Cross-reactivity
The negative blood specimen was spiked with serum specimens of infectious diseases and then tested according to the standard procedure. The results showed that the CADY Malaria Pf/Pan Ag Rapid Test had no cross-reaction with the following tested serum specimens of infectious disease.

Specimen	Sample Size	Pf Reactivity	PV Reactivity
Filariasis Serum	10	Negative	Negative
Typhoid Serum	10	Negative	Negative
Dengue Ns1 Ag Serum	10	Negative	Negative
HBS Ag Serum	10	Negative	Negative
ANA Serum	10	Negative	Negative
RF (S2,500IU/ml)	10	Negative	Negative

3. Precision
Within run and between run precisions have been determined by testing 20 replicat es with four categories of the specimens: negative, weak, medium and strong positi ve specimens. The negative, weak, medium and strong positive specimens were corr ectly identified in all of the tests performed in each run.

4. Interference
Common substances (such as pain and fever medication, blood components) may affect the performance of the CADY Malaria Pf/Pan Ag Rapid Test. This was studied by spiking of these substances to the three levels of the pHRP-II and pLDH standard control. The results are presented in the following table and demonstrate that the substances studied did not affect the performance of the CADY Malaria Pf/Pan Ag Rapid Test. Note: -: Negative; +: Weak positive; +++: Strong positive.

Potential Interfering Substance Spiked	Negative	Weak Positive	Strong Positive	Negative	Weak Positive	Strong Positive
Control	—	+	+++	—	+	+++
Billrubin 20mg/dl	—	+	+++	—	+	+++
Creatnine 442 umol/L	—	+	+++	—	+	+++
Glucose 55mmol/L	—	+	+++	—	+	+++
Alloumin 60g/L	—	+	+++	—	+	+++
Salicylic acid 4.34 mmol/L	—	+	+++	—	+	+++
Heparin 3,000 U/L	—	+	+++	—	+	+++
EDTA 3.48 uMOI/L	—	+	+++	—	+	+++
Human IgG 150mg/dl	—	+	+++	—	+	+++

5.Limit of Detection
The minimum detection limit for malaria worldwide parasite panel is up to 50P/ul and Cady Malaria Pf/Pv Antigen Rapid test detected up to 50p/ul in all the parasite panel which included USF Benin-1,USF ph-1 03,USF Nigeria XII, USF Santa Lucia, 5-FC 27/A3.

Limitation of Test.

1. The Assay Procedure and the Interpretation of Assay Result sections must be followed closely when testing for the presence of plasmodium antigen in whole blood from individual subjects. Failure to follow the procedure may lead to inaccurate test results.
2. The CADY Malaria Pf/Pan Ag Rapid Test is limited to the qualitative detection of plasmodi um antigen in whole blood. The intensity of the test line does not have linear correlation with the antigen titer in the specimen.
3. In the case that both Pan and Pf lines are visible, interpret the result cautiously. Infection by Pf alone or co-infection with Pf and any of the other three plasmodium species co uld results in color development on both Pan and Pf lines. Thus, when both Pan and Pf lines are visible, follow up with appropriate additional testing methods for further discrimination of plasmodium species present in the sample.
4. A negative result for an individual subject indicates absence of detectable plasmodium antig en. However, a negative test result does not preclude the possibility of exposure to or infection with plasmodium.
5. A negative result can occur if the quantity of the plasmodium antigen present in the specim en is below the detection limits of the assay, or the antigens that are detected are not present during the stage of disease in which a sample is collected.
6. A result positive for pLDH and negative for pHRP-II does not necessarily rule out a Pf infe ction, since, due to the genetic diversity some Pf isolates lack the HRP-II gene7,8.
7. Infection may progress rapidly. If the symptom persists, while the result from CADY Malar ia Pf/Pan Ag Rapid Test is negative or non-reactive, it is recommended to test with an alterna tive test method.
8. Some specimens containing an unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
9. The results obtained with this test should only be interpreted in conjunction with other diag nostic procedures and clinical findings.

Symbol	Meaning	Symbol	Meaning	Symbol	Meaning	Symbol	Meaning
LOT	Batch Number		Keep Dry	REF	Catalog#		Manufacturer
IVD	In Vitro Diagnostic Use	CE	CE Mark		Expiry Date	EC REP	EU Authorized Representative
	Do not Reuse		See Instructions For Use		Store between 2-30 C		Manufacturing Date
	Keep away from sunlight						

IFU No : R/IFU/RDT/M/02
Issue Date : 01/09/2022
Rev No : 02

Revital®

CADY®

Malaria Pf/Pan Ag Rapid Test

[Package and Specification]

25 Test/box (1 test x 25 Pouches)

Intended Use

The CADY Malaria Pf/Pan Ag Rapid Test is a lateral flow chromatographic immunoassay for the simultaneous detection and differentiation of Plasmodium falciparum (Pf) antigen and P. vivax (Pv), P. ovale (Po), or P. malariae (Pm) antigen in human blood specimen. This device is intended to be used by professionals as a screening test and provides a preliminary test result to aid in the diagnosis of infection with plasmodium.

Any use or interpretation of this preliminary test result must also rely on other clinical findings and the professional judgment of health care providers. Alternative test method(s) should be considered to confirm the test result obtained by this device.

Intended User

1.Pathology Labs
2.Blood Banks
3.Hospitals
4.Individual users

Summary and explanation of the Test

Malaria is a mosquito-borne, hemolytic, febrile illness that infects over 200 million people and kills more than 1 million people per year. It is caused by four species of Plasmodium: P. falciparum, P.vivax, P. ovale, and P. malariae. All Plasmodium spp. infect and destroy human erythrocytes and lead to chills, fever episodes, anemia, and splenomegaly. P. falciparum causes more severe disease than the other Plasmodium species and accounts for most malaria deaths. P. falciparum and P. vivax are the most common pathogens, however, there is considerable geographic variation in species distribution. Traditionally, malaria is diagnosed by the demonstration of the organisms on Giemsa stained thick smears of peripheral blood, and the different species of Plasmodium are distinguished by their appearance in infected erythrocytes 1.

The technique is performed only by well-trained microscopists using defined protocols2, which presents major obstacles for the remote and poor areas of the world. The CADY Malaria Pf/Pan Ag Rapid Test is developed for solving these obstacles. The test utilizes a pair of antibodies to detect P. falciparum Histidine-rich protein II (pHRP-II), and a pair of antibodies to detect the plasmodium Lactate Dehydrogenase (pLDH) for detection of P. falciparum, P. vivax, P. ovale and P. malariae, thus enabling simultaneous detection and differentiation of an infection with P. falciparum and/or any of the other three plasmodium species 3-6. It can be performed within 30 minutes by minimally skilled personnel without the use of laboratory equipment.

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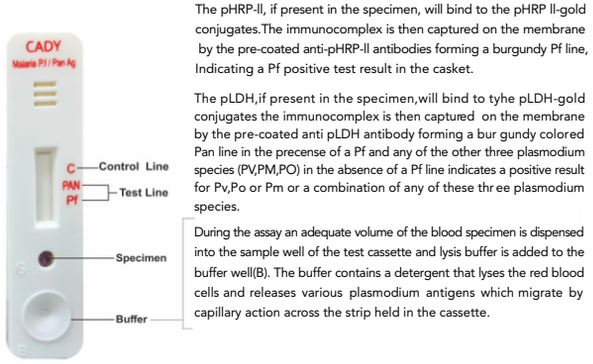
Performance Characteristics

The CADY Malaria Pf/Pan Ag Rapid Test is a lateral flow chromatographic immunoassay. The strip in the test cassette consists of:

1) a burgundy colored conjugate pad containing monoclonal anti-pHRP-II antibody conjugated with colloidal gold (pHRP-II-gold conjugates), monoclonal anti-pLDH antibody conjugated with colloidal gold (pLDH-gold conjugates) and a control antibody conjugated with colloidal gold and 2) a nitrocellulose membrane strip containing two test lines (Pan and Pf lines) and a control line (C line). The Pan line is pre-coated with anti-pLDH antibody for the detection of infection with any of the four species of plasmodium, the Pf line is pre-coated with antipHRP- II antibodies for the detection of Pf infection, and the C line is coated with a controlline antibody. Absence of any test lines (Pan and Pf) suggests a negative result. Thetest contains an internal control (C line) which should exhibit a burgundy colored line of the immunocomplex of the controlantibodies regardless of the color development on any of the test lines. If the C line does not develop, the test result is invalid, and the specimen must be retested with another device.



The pHRP-II, if present in the specimen, will bind to the pHRP II-gold conjugates.The immunocomplex is then captured on the membrane by the pre-coated anti-pHRP-II antibodies forming a burgundy Pf line, Indicating a Pf positive test result in the casket.

The pLDH,if present in the specimen,will bind to tyhe pLDH-gold conjugates the immunocomplex is then captured on the membrane by the pre-coated anti pLDH antibody forming a bur gundy colored Pan line in the precense of a Pf and any of the other three plasmodium species (PV,PM,PO) in the absence of a Pf line indicates a positive result for Pv,Po or Pm or a combination of any of these thr ee plasmodium species.

During the assay an adequate volume of the blood specimen is dispensed into the sample well of the test cassette and lysis buffer is added to the buffer well(B). The buffer contains a detergent that lyses the red blood cells and releases various plasmodium antigens which migrate by capillary action across the strip held in the cassette.

Kit Components

1. 25 Individually sealed foil pouches containing:
a. One cassette device
b. One desiccant
c. 5 µL blood transfer devices
2. Blood lysis buffer (3 mL/bottle)
3. One package insert (instruction for use)

Materials Required But Not Provided

1.Clock or Timer
2. Safety lancet

Warnings and Precautions

For in Vitro Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not open the sealed pouch, unless ready to conduct the assay
3. Do not use expired devices.
4. Bring all reagents to room temperature (15-30 °C) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Hemolyzed blood may be used for the testing, but do not use precipitants.
7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
10. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
11. Handle the Negative and Positive Control in the same manner as patient specimens.
12. The testing results should be read 20-25 minutes after a specimen is applied to the sample well of the device. Any results interpre ted outside 30 minutes should be considered invalid and must be repeated.
13. Do not perform the test in a room with strong air flow, i.e. an electric fan or strong airconditioning.

Reagent Preparation and Storage Instructions

All reagents are ready to use as supplied. Store unused test device unopened at 1-30 °C. If stored at 2-8 °C, ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit to temperatures above 30 °C.

Specimen Collection and Handling

Consider any materials of human origin as infectious and handle them with standard bio-safety procedures.
Drops of whole blood can be obtained by either finger tip puncture or venipuncture. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer).
Whole blood specimen should be stored at 2-8 °C for up to 3 days if not tested immediately. The specimen should be frozen at -20 °C for longer storage. Avoid multiple freeze-thaw cycles.

Assay Procedure

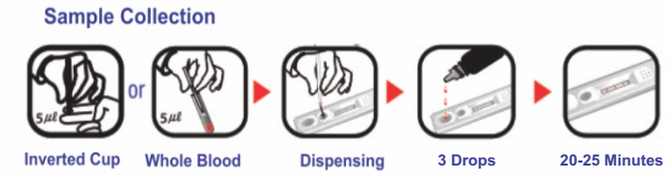
Step 1: Bring the specimen and test components to r oom temperature if refrigerated or frozen. Once the specimen is thawed, mix well prior to performing the assay. Blood will be hemolyzed after thawing.

Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

Step 3: Fill the blood transfer device (inverted Cup) with the blood specimen as shown in the following images. The volume of the specimen around 5ul

Note: Practice a few times prior to testing if you are not familiar with the blood transfer device. For better precision, transfer specimen by pipette capable of delivering a 3 Ml volume. Holding the blood transfer device vertically, dispense the entire specimen into the center of the sample well (S well) making sure that there are no air bubbles. Then immediately add 3 drops of Blood
Lysis Buffer (100-120 µL) into center of the buffer well (B well)

Sample Collection



Step 5: Set up timer.

Step 6: Results can be read at 20 - 25 minutes. It may take more than 20 minutes to have the background become clearer. However, results must be confirmed at the end of the 25 minutes only. Any results interpreted outside 30 minutes should be considered invalid and must be repeated.

Discard used device after interpreting the result following local laws governing the disposal of device.

Quality Control

1. Internal Control: This test contains a built-in control feature, the C line. The C line develops after adding specimen extract. Otherwise, review the whole procedure and repeat test with a new device.
2. External Control: Good Laboratory Practice recommends using external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:

a. A new operator uses the kit, prior to performing testing of specimens.
b. A new lot of test kit is used.
c. A new shipment of kits is used.
d. The temperature during storage of the kit falls outside of 1-30 °C.
e. The temperature of the test area falls outside of 15-30 °C.
f. To verify a higher than expected frequency of positive or negative results.
g. To investigate the cause of repeated invalid results.