THE UNITED REPUBLIC OF TANZANIA

MINISTRY OF HEALTH

TANZANIA MEDICINES AND MEDICAL DEVICES AUTHORITY

PUBLIC ASSESSMENT REPORT FOR SMART MALARIA P.F/ PAN AG RAPID TEST

Version number v2.0, 09-09-2024

TMDA Headquarters, Plot No. 56/1, Block E, Kisasa B Centre, Swaswa Road, P. O. Box 1253, Dodoma – Tanzania, Telephone: +255 (26) 2961989/2061990/+255 (22) 2450512/2450751/2452108, Email: info@tmda.og.tz, Website: **www.tmda.go.tz**

Toll free: 0800110084

1. Introduction

Smart RDT is a class *C in-vitro diagnostic device* belonging to the *Clinical laboratory* specialty category. It is approved in Tanzania as a *kit comprises of one (1) test Cassette, and one (1) dropper in aluminum pouch, with buffer, and desiccant* and for use in the *Diagnosis of Malaria* by *healthcare professionals.*

1.1. Administrative Information

Registration number	TAN 23 MDR 0278
Brand Name (if relevant)	Smart Rapid Diagnostic Test (RDT)
Common name	Malaria P.F/Pan Ag Rapid Test Cassette
Class of the device and rule applied	Class C according to rule number 3 of classification of in-vitro diagnostic devices
GMDN code and term	52311- Multiple Plasmodium species antigen IVD, kit, rapid ICT, clinical
Name and complete address of the Market Authorization Holder	KS Global Business Enterprises (T) Ltd P.O. Box 71852, DAR ES SALAAM TANZANIA Tel: +255 784 222 883 Email: ksglobal2015@yahoo.com
Name and address(es) of local responsible person (LRP).	KS Global Business Enterprises (T) Ltd P.O. Box 71852, DAR ES SALAAM TANZANIA Tel: +255 784 222 883 Email: ksglobal2015@yahoo.com

1.2. Assessment Procedure

The application for registration of *Smart RDT* was submitted on 27/10/2022. The product underwent *full* assessment. Assessment was completed in *four (4)* rounds of evaluation. *Smart RDT* was registered on *04-12-2023*.

2. Technical information

2.1. Intended use

The intended use of *Smart RDT* as declared by the manufacturer and approved by TMDA is *"used to detect and differentiate between Plasmodium falciparum and other Plasmodium species in human blood, aiding in the diagnosis of malaria". Smart RDT* is approved for use in *healthcare settings* by *trained professionals only.*

2.2. Device details and features

Smart RDT is a diagnostics kit with two (2) components. Smart RDT has been registered as a *Malaria Rapid Diagnostic Kit* which consists of *one (1) test Cassette, and one (1) dropper.*

Smart RDT is a *rapid diagnostic kit which is manually operated*. It is used to *aid diagnosis* of *Malaria*. *Smart RDT (Malaria Pf/Pan Ag Rapid Test)* operates by *detecting specific proteins from malaria parasites in a blood sample. When the sample is placed on the test strip with a buffer, red blood cells break apart, releasing these proteins. If Plasmodium falciparum is present, the test detects HRP-II (Histidine-rich protein II), forming a coloured line at T2. For other malaria species (P. vivax, P. malariae, P. ovale), the test detects pLDH (Plasmodium lactate dehydrogenase), forming a line at T1. A control line (C) ensures the test is working correctly. No lines at T1 or T2 indicate a negative result.* The test out-put is *qualitative.*

The type of specimen used is *Whole blood* which can be collected through *Capillary or Venipuncture route*.

Product Features

Test Cassette:	*Made of medical-grade plastic, the test cassette houses the internal components. It includes designated wells for sample and buffer application.*
Membrane Strip:	*The core component is a nitrocellulose membrane strip, which is pre-coated with mouse monoclonal antibodies specific to HRP-II (for Plasmodium falciparum) and pLDH (for other Plasmodium species). This membrane facilitates the capillary action for the test reaction.*
Conjugate Pad:	*Contains a mixture of colloidal gold particles bound to monoclonal antibodies for HRP-II and pLDH. This allows the detection of antigens in the blood sample when they bind to the corresponding antibodies.*
Absorbent Pad:	*Located at the end of the test strip, it absorbs excess sample and buffer to control the flow of reagents across the membrane.*
Buffer Solution:	*A lysis buffer included with the kit, containing detergent to break down red blood cells and release malaria antigens into the test system.*
Pipette Dropper:	*A disposable plastic dropper provided to accurately collect and dispense the blood sample into the sample well.*
Desiccant:	*Included in the packaging to maintain dry conditions and protect the test components from moisture, ensuring stability during storage.*

Control Line: *An integrated control feature in the cassette ensures the validity of the test by displaying a coloured line when the test is functioning correctly.*

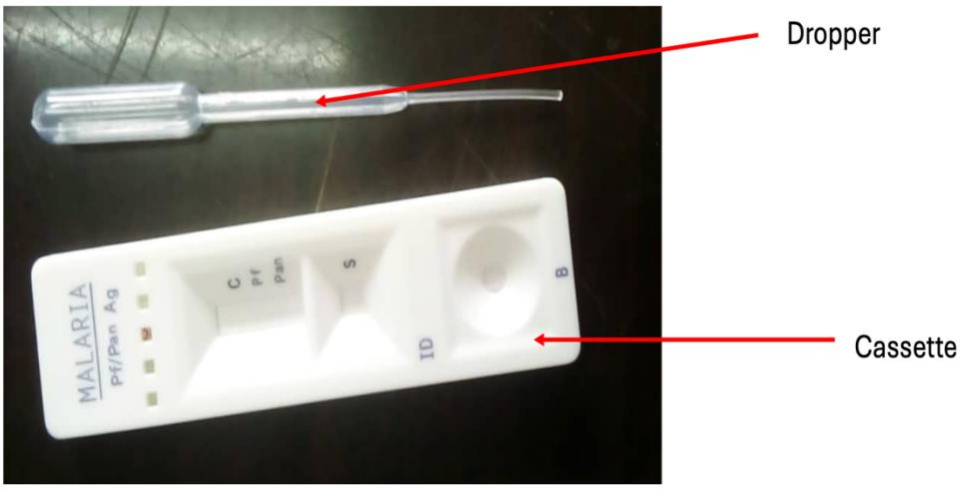


Figure 1*: Actual product picture with dropper, both available in aluminum pouch*

2.3. Commercial presentation

There is *one (1)* approved commercial presentations as follows: in each primary pack there is *one (1) Smart RDT cassette, and one (1) dropper in aluminum pouch.* ***25*** *aluminum pouch with cassette and dropper together with buffer and desiccant placed in* ***carton box*** *as* a secondary pack.

Additional contents include Package Insert

2.4. Items required but not submitted

Common clinical medical devices needed for whole blood collection procedure, example blood lancet, or blood collection needle, etc.

3. Storage instructions

3.1.1. Shelf-life

The approved shelf-life is *24* months.

3.1.2. Storage conditions

The recommended storage conditions as *stated in the IFU is "All reagents are ready to use as supplied. Store unused test device unopened, preferably at 2°C-30°C. Do not expose the kit over 30°C. Do not freeze the kit. 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 if it is stored at 2°C-30°C".*

3.1.3. Shipping conditions

The manufacturer did not declare shipping conditions.

4. Manufacturing site audit

The manufacturer of the device is:

Zhejiang Orient Gene Biotech Co., Ltd
3787#, East Yangguang Avenue, Dipu Street,
Anji 313300, Huzhou, Zhejiang, China
Website: www.orientgene.com

Quality audit of the manufacturing facility was conducted through *site visit* on 20th to 21st March 2019. The site was found to be compliant to ISO 13485 requirements.

5. Performance Evaluation

5.1. Analytical Performance

The analytical performance characteristics of the Smart - Malaria Pf/Pan Ag Rapid Test Cassette (Whole Blood) were established through the following test parameters:

i. Accuracy (Trueness)
ii. Precision (Repeatability and Reproducibility)
iii. Analytical Sensitivity
iv. Analytical Specificity

The evaluation considered various environmental conditions and blood specimen types (finger prick and venous EDTA), confirming high sensitivity and specificity under these conditions.

5.2. Clinical Performance

Clinical performance was conducted at the Ifakara Health Institute, Bagamoyo and Kibiti districts in Tanzania. The following parameters were tested:

i. Sensitivity
ii. Specificity
iii. Usability (ease of use and interpretation)
iv. Comparison to reference methods (microscopy and qPCR)

Based on the results of the performance studies, it was concluded that the test *sensitivity for Plasmodium falciparum detection using finger prick samples was 93.94%, while 94.95% when using venous EDTA samples and the sensitivity for detecting other Plasmodium species was 94.44% with finger prick blood and 95.45% with venous EDTA blood. Similarly, the specificity for detecting Plasmodium falciparum was 99.1% with finger*

prick blood and 98.65% with venous EDTA blood and the specificity for detecting other Plasmodium species was 97.3% with finger prick blood and 97.75% with venous EDTA blood. The studies further concluded that the Malaria Pf/Pan Ag Rapid Test Cassette (Whole Blood) is capable of consistently producing accurate and reliable test output in diverse conditions and environments.

6. Product label and instructions for use

The content of the primary and secondary labels is in line with TMDA labeling requirements in terms of content, layout and design. The label contains sufficient information for proper identification of the device and post marketing follow up of the product in the market.

The *instruction for use* includes all the relevant information to ensure correct and safe use of the device by intended user.

6.1. Primary pack

物料名称:	KS Pouch
尺寸:	120*60mm
物料编码:	B11093-01
材质:	PET12/AL17/CPPS8
工艺:	彩色印刷
日期:	2020-5-12

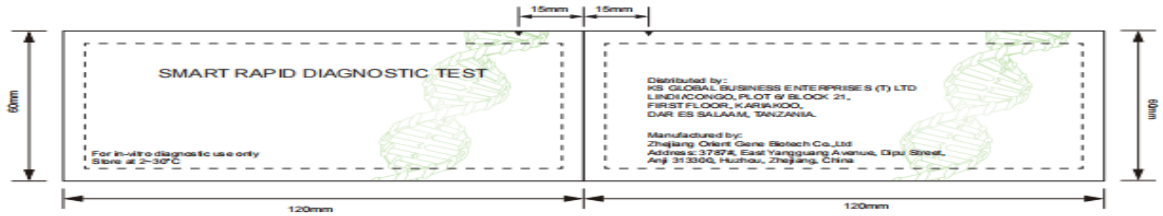


Figure 2: *The mock-up labeling of the aluminum pouch*

6.2. Secondary pack

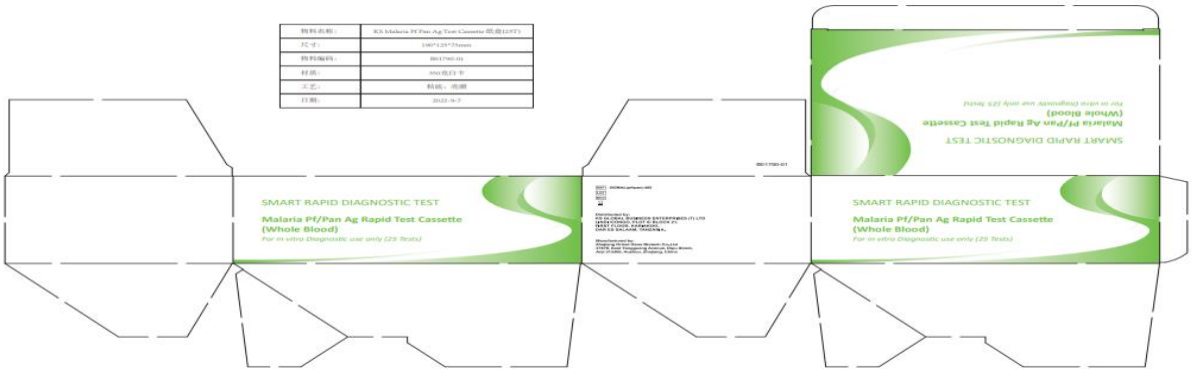


Figure 3: *The secondary mock-up labeling.* 6.3 Instructions for use/Package insert

Instructions for use can be accessed by clicking here

7. Risk – Benefit Analysis

On basis of the data submitted, the current state of knowledge and compliance of the manufacturer to ISO 13485, the benefit of the product outweighs the risks associated with its use when used in accordance to the manufacturer instruction. Smart RDT was recommended for registration.

8. Post-approval updates

8.1. Variation applications

Reference number	Date submitted	Change requested	Recommendation	Granting date
NA	NA	NA	NA	NA

8.2. Feedback from pharmacovigilance, post marketing surveillance and enforcement activities

Type of feedback	Impact	Response
No recorded Adverse Event	NA	NA

8.3. Re-registration applications

NA

CHANGE HISTORY

Version number	Date	Description of update	Section(s) Modified	Approval date

Malaria P.f./Pan Ag Rapid Test Cassette (Whole Blood)

INTENDED USE

The Malaria P.f./Pan Ag Rapid Test Cassette (Whole Blood) is a rapid lateral flow chromatographic immunoassay for the simultaneous detection and differentiation of HRP-II (Histidine-rich protein II) specific to plasmodium falciparum and pLDH (plasmodium lactate dehydrogenase) specific to plasmodium species (Pan) in human blood specimen as an aid in the diagnosis of Malaria infection. It is for *in vitro* diagnostic use only.

SUMMARY

Malaria is a serious, sometimes fatal, parasitic disease characterized by fever, chills, and anemia and is caused by a parasite that is transmitted from one human to another by the bite of infected Anopheles mosquitoes. There are four kinds of malaria that can infect humans: Plasmodium falciparum, P. vivax, P. ovale, and P. malariae. In humans, the parasites (called sporozoites) migrate to the liver where they mature and release another form, the merozoites. The disease now occurs 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. At the present, malaria is diagnosed by looking for the parasites in a drop of blood. Blood will be put onto a microscope slide and stained so that the parasites will be visible under a microscope.

PRINCIPLE

The Malaria P.f./Pan Ag Rapid Test Cassette (Whole Blood) contains a membrane, which is precoated with mouse monoclonal antibodies specific to HRP-II of P. falciparum on test line Pf region and with mouse monoclonal antibodies specific to lactate dehydrogenase of Plasmodium species (P. falciparum, P. vivax, P. malariae and P. ovale) on test line Pan region respectively. A conjugate pad is dispensed with a mixture of mouse monoclonal antibodies specific to HRP-II of P.f and mouse monoclonal antibodies specific to pLDH of pan - colloidal gold.

During the assay, an adequate volume of the blood specimen is dispensed into the sample well (S) of the test cassette, a lysis buffer is added to the buffer well (B). The buffer contains a detergent that lyses the red blood cells and releases various antigens, which migrate by capillary action across the strip held in the cassette. Pan-LDH if presents in the specimen will bind to the Pan-LDH-gold conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-Pan-LDH antibody, forming a burgundy colored Pan band, indicating a Panpositive test result.

Alternatively, pHRP-II if presents 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 colored Pf band, indicating a Pf positive test result.

Absence of any T bands suggests a negative result. The test contains an internal control (C band) which should exhibit a burgundy colored band of the immunocomplex of goat anti- mouse IgG / mouse IgG (anti-Pan-LDH and anti-pHRP-II)-gold conjugates regardless of the color development on any of the T bands. Otherwise, the test result is invalid and the specimen must be retested with another cassette.

MATERIALS PROVIDED

1.Test cassettes 2. Droppers 3. Buffer 4. Package insert

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or timer
2. Collection by venipuncture: collection tube (containing EDTA, citrate or heparin)
3. Collection using a lancet: sterile lancet

PRECAUTIONS

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- The instruction must be followed exactly to get accurate results. Failure to follow the insert gives inaccurate test results.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout testing and follow the standard procedures for proper disposal of specimens.
- Hemolized blood may be used for the testing, but do not take precipitants.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested.
- Humidity and temperature can adversely affect results.
- Do not perform the test in a room with strong air flow, ie. an electric fan or strong airconditioning.

STORAGE AND STABILITY

All reagents are ready to use as supplied. Store unused test cassette unopened, preferably at 2°C-30°C. Do not expose the kit over 30°C. Do not freeze the kit. Ensure that the test cassette is brought to room temperature before opening. The test cassette is stable through the expiration date printed on the sealed pouch if it is stored at 2°C-30°C.

SPECIMEN COLLECTION AND PREPARATION

Collection by venipuncture:

- Collect whole blood into a collection tube (containing EDTA, citrate or heparin) by venipuncture.
- If specimens are not immediately tested, they should be refrigerated at 2 ~ 8°C. For storage periods greater than three days, freezing is recommended. They should be brought to room temperature prior to use. Using the specimen after long-term storage of more than three days can cause non-specific reaction.
- When stored at 2 ~ 8°C, the whole blood sample should be used within three days.

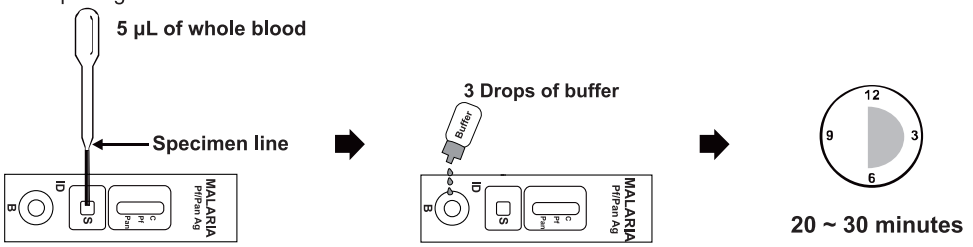
Collection using a lancet:

- Clean the area to be lanced with an alcohol swab.
- Squeeze the end of the fingertip and pierce with a sterile lancet.
- Wipe away the first drop of blood with sterile gauze or cotton.
- Using the dropper provided, while gently squeezing the tube, immerse the open end in the blood drop and then gently release the pressure to draw blood into the dropper.

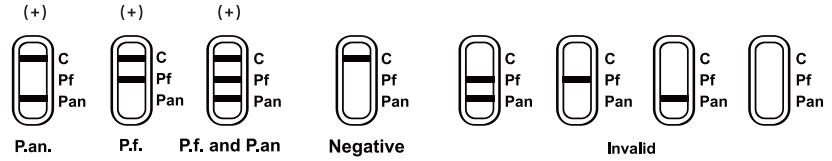
TEST PROCEDURE

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

1. Remove the test cassette from the foil pouch and use it as soon as possible. Best results will be obtained if the assay is performed within one hour.
2. Place the test cassette on a clean and level surface. Be sure to label the cassette with specimen's ID number.
3. With a 5 µL mini plastic dropper provided, draw whole blood specimen to exceed the specimen line as showed in the following image and then transfer drawn whole blood into the sample well (S). Then add 3 drops (about 120 µL) of Lysis Buffer to the buffer well (B) immediately.
   Note: Practice a few times prior to testing if you are not familiar with the mini dropper. For better precision, transfer specimen by pipette capable to deliver 5 µL of volume.
4. Set up timer.
   If preferred, after 5 minutes of adding specimen and buffer, you may add one more drop of Lysis Buffer to help the background become clearer.
5. Results can be read in 20 to 30 minutes. It may take more than 20 minutes to have the background become clearer. Don't read results after 30 minutes. To avoid confusion, discard the test cassette after interpreting the result.



INTERPRETATION OF RESULTS



POSITIVE:

P. f or mixed malaria Positive: One line appears in the control region; one line appears in Pan line region and one line appears in P.f. line region.

P.f Positive: One line appears in the control region, and one line appears in P.f. line region.

P.v or P.m or P.o Positive: One line appears in the control region and one line appears in Pan line region.

NEGATIVE: Only one colored line appears in the control region.

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

*** Note:** pLDH is Pan specific to the lactate dehydrogenase of Plasmodium species (P.f, P.v, P.o, P.m).

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 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.

LIMITATION

1. The Malaria P.f./Pan Ag Rapid Test Cassette (Whole Blood) is for 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. The Malaria P.f./Pan Ag Rapid Test Cassette (Whole Blood) 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 known relevant interference, haemolytic samples, rheumatoid factors-contained samples and lipaemic, icteric samples can lead to impair the test results.
3. In a few cases, where the Pf band is positive and the Pan band is negative, it may indicate a case of post treatment malaria. However, such a reaction pattern may also be obtained in a few cases of untreated malaria. Retesting after 2 days is advised, in such cases.
4. The test is limited to the detection of antigen to Malaria Plasmodium sp. Although the test is very accurate in detecting HRP-II specific to P. falciparum or pLDH specific to plasmodium species (P. falciparum, vivax, malariae, ovale), a low incidence of false results can occur. Other clinically available tests are required if questionable results are obtained.
5. 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.
6. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

PERFORMANCE CHARACTERISTICS

1. Clinical Performance for P.f Ag test:

A total of 352 samples from susceptible subjects were tested by the Malaria P.f./Pan Ag Rapid Test Cassette (Whole Blood) and by thick blood smear test.

Method		Smear Test		Total Results
Malaria Pf/Pan Ag Rapid Test	Results	Positive	Negative	
	Positive	50	4	54
	Negative	0	298	298
Total Results		50	302	352

Relative Sensitivity: 100%

Relative Specificity: 98.7%

Overall Agreement: 98.9%

2. Clinical Performance for Pan Ag test:

A total of 289 samples from susceptible subjects were tested by the Malaria P.f./Pan Ag Rapid Test Cassette (Whole Blood) and by thick blood smear test.

Method		Smear Test		Total Results
Malaria Pf/Pan Ag Rapid Test	Results	Positive	Negative	
	Positive	63	3	66
	Negative	0	223	223
Total Results		63	226	289

Relative Sensitivity: 100%

Relative Specificity: 98.7%

Overall Agreement: 99.0%

3. **Precision:** Within-run and between-run have been determined by the testing 10 replicates of four specimens: a negative, a low positive, a medium positive and a strong positive. All values were correctly identified 100% of the time.
4. **Interference:** To evaluate the interference of Malaria Pf/Pan Ag Rapid Test with known relevant interfering specimens, the haemolytic samples, rheumatoid factors-contained samples and lipaemic, icteric samples were investigated. In these studies, those specimens did not interfere with the Malaria Pf/Pan Ag Rapid Test.

REFERENCES

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2. David L. Vander Jagt, Lucy A. Hunsaker and John E. Heidrich: Partial Purification and Characterization of Lactate Dehydrogenase from Plasmodium falciparum. Molecular and Biochemical Parasitology, 4 (1981) 255-264.

3. David J. Bzik, Barbara A, Fox and Kenneth Gonyer: Expression of Plasmodium falciparum lactate dehydrogenase in Escherichia coli Molecular and Biochemical Parasitology, 59(1993) 155-166.

4. Histidine-Rich Protein II: a Novel Approach to Malaria Drug Sensitivity Testing ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 2002, p. 1658°©1664 Vol. 46, No.

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