

	Anti-Malaria Campaign (AMC)					
SOP for malaria Rapid Diagnostic Test Kits						
SOP title:	Performing malaria Rapid Diagnostic Test Kits					
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Approved:				Date	10.03.2015	

1. BACKGROUND:

Malaria Rapid Diagnostic Test (RDT) Kits can be used as a supplementary measure to microscopy to provide rapid diagnosis of malaria.

2. PURPOSE:

This Standard Operating Procedure outlines the procedure of performing a malaria Rapid Diagnostic Test.

3. SCOPE:

This procedure applies to all Laboratory Technicians using malaria Rapid Diagnostic Test Kits provided by the Anti Malaria Campaign.

4. Policy:

This procedure shall be reviewed whenever a new stock of malaria Rapid Diagnostic Test Kits is procured. Any deviation from the procedures outlined shall be recorded and reported.

5. REQUIREMENTS (Figure 1):

- 5.1 Malaria Rapid Diagnostic Test Kits (Carestart™ Malaria HRP2/pLDH (Pf/PAN) Combo)
- 5.2 Sterile lancets
- 5.3 Alcohol swab
- 5.4 Pipette (provided with the test kits with a marking at 5 µL level; or a micropipette to get 5 µL of blood)
- 5.5 Assay buffer
- 5.6 Puncture resistant container (sharps bin)
- 5.7 Infectious and Non- Infectious bins
- 5.8 All-purpose disinfectant
- 5.9 Laboratory gown/ coat
- 5.10 Gloves
- 5.11 Soap/liquid hand wash
- 5.12 Instruction leaflet
- 5.13 H/AMC/P1 form
- 5.14 RDT Result Report form
- 5.15 Pen
- 5.16 Timer or clock



Figure 1: Components in a Box of CareStart™ RDT Test Kit

1. assay buffer
2. blood lancet
3. alcohol pad
4. pipette
5. test kit (in aluminum pouch)

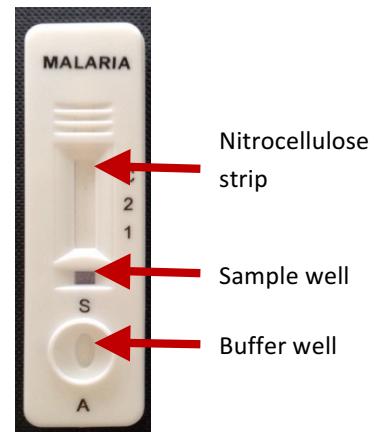


Figure 2: Parts of the RDT

6. Description of the RDT:

CarestartTM Malaria HRP2/pLDH (Pf/PAN) Combo from ACCESS BIO (CAT NO. G0131) is the RDT presently distributed free of charge by the Anti Malaria Campaign for rapid diagnosis of malaria.

The test kit is designed to detect 2 parasite antigens found in infected blood, namely parasite Lactate Dehydrogenase (pLDH) present in asexual and sexual stages of *Plasmodium vivax*, *P. falciparum*, *P. ovale* and *P. malariae* and Histidine Rich Protein2 (HRP2) present only in *Plasmodium falciparum* (trophozoites & immature gametocytes). pLDH is an enzyme found only in live parasites and therefore is related to the peripheral parasite density and is cleared from blood stream soon after parasite clearance (about 2 days). HRP2 is a water soluble persisting antigen that is found in the circulating peripheral blood.

7. Parts of the RDT (Figure 2)

RDT test kit contains the following components.

- Sample well (denoted by S in the test kit)
- Buffer well (denoted by A in the test kit)
- Nitrocellulose strip (showing test and control lines)

8. Testing Procedure

8.1. Record information of the patient data in a H/AMC/P1 form (*Serial No., date, name, age, sex, address and history of fever*)

8.2. Collect all the items required to perform the test. If the RDTs have been refrigerated, it is better to get the RDT to the room temperature.

8.3. Take out the test kit from the sachet and keep it on a flat leveled surface.

8.4. Write on the test kit with a marker pen, pen or a pencil, the name of the patient, date.

8.5. Collect blood for the test.

8.5.1. If finger prick blood is used

8.5.1.1. Holding patient's left hand palm upwards, select the third finger from the thumb. (The big toe can be used with infants).

8.5.1.2. Clean the finger with a piece of cotton soaked with 70% alcohol, using firm strokes to remove grease and dirt from the ball of the finger. Puncture the ball of the finger with a sterile lancet, using a quick rolling action.

- 8.5.1.3. Apply gentle pressure to the finger to express the first drop of blood and wipe it away with a dry piece of cotton wool. (Make sure that no strands of cotton remain on the finger to be later mixed with the blood.)
 - 8.5.1.4. Take a sample pipette and while gently squeezing the tube immerse the open end in the blood drop and then gently release the pressure to draw blood into the sample pipette up to the black line.
- 8.5.2. If anticoagulated blood is used, collect 5 μ l of blood using the sample pipette provided (up to the black line) or with a micropipette.
- 8.6. Add 5 μ l of blood to the sample well (indicated by S).
- 8.7. Add two drops of (60 μ l) of assay buffer into the buffer well (indicated by A) and write down the time of adding the buffer to the buffer well (preferably on the RDT).
- 8.8. Read the result in 20 minutes. (Since non specific binding can occur after 20 minutes, care should be taken to examine the test kit in 20 minutes).
- 8.9. Write the result as interpreted according to the steps given in Interpretation section (#11).
- 8.10. Keep the RDT for further reference.

9. STORAGE and TRANSPORT

- 9.1. Store and transport the RDTs within the temperature range of 1°-40°C (34°-104°F). RDTs can be stored within the main component of the refrigerator, It should never be kept in freezer compartment.
- 9.2. If the test kit is stored in a refrigerator, the device need to be equilibrate to room temperature prior to use.

10. SAFETY RULES

- 10.1. Read provided instructions for use before using the test kit.
- 10.2. Do not use components from other lots. However, buffer solutions and other items can be used with the RDTs of the same lot which are provided later.
- 10.3. Do not use the RDT if the package is damaged.
- 10.4. Use the test device and optional components (lancet/alcohol pad) immediately after opening its package.
- 10.5. Use disposable protective gloves while performing the test.
- 10.6. Keep out of reach of children.

11. INTERPRETATION

RDT should be examined under clear light for interpretation, since sometimes faint control and test lines may be visible. Interpretation of results should be done in 20 minutes as follows (figure 3).

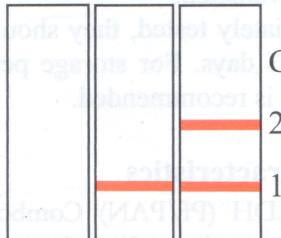
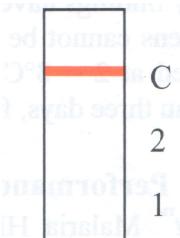
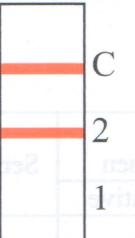
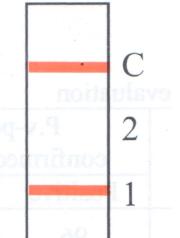
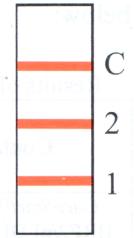
<u>Invalid</u>	<u>Negative</u>	<u>PAN Positive</u>	<u><i>P. falciparum</i> Positive</u>	<u>Positive <i>P. falciparum</i> mono infection or mixed infection</u>
Test should be considered invalid if the line in the control area (C) does not appear. Other lines may or may not be present. The test should be repeated	Only the line in the control area (C) is present.	The line in the control area (C) and the Pan specific line (line 2) are present. This indicates a positive result for <i>P. vivax</i> , <i>P. falciparum</i> , <i>P. ovale</i> or <i>P. malariae</i> .	The line in the control area (line C) and the HRP specific line (line 1) are present.	Control line (C) and the two test lines (line 1 and 2) are present. A blood smear has to be examined for species confirmation
 1) Invalid	 2) Negative	 3) Positive (PAN Specific)	 4) Positive (<i>P. falciparum</i>)	 5) Mixed infection (<i>P. falciparum</i> or Mixed infection)
If malaria is highly suspected, <ul style="list-style-type: none"> - Repeat the test (as very low <i>P. vivax</i> and <i>P. falciparum</i> parasitaemia may give a negative result) - Perform microscopy (as <i>P. malariae</i> and <i>Plasmodium ovale</i> may fail to give a positive result) 	Generally if <i>P. falciparum</i> is present HRP2 line (line 1) is also visible, however certain <i>P. falciparum</i> strains do not have the HRP2 antigen. Such strains will show only the pLDH line. Also in <i>P. falciparum</i> hyper parasitaemic conditions, due to prozone effect, the HRP2 line may not appear initially. In such instances it may appear in follow up tests.	Since HRP2 antigen is a water soluble antigen secreted by the parasite and found in circulating peripheral blood, this line will appear even when parasites are sequestered. (In this instance a blood smear could give a negative result) HRP2 may persist up to 3 weeks after parasite clearance and hence could give a false positive result even after complete parasite clearance.		

Figure 3 -Interpretation of the CareStart™ Rapid Diagnostic Test