**Model SOP**

**Standard Operating Procedure**

**Name of the facility / activity : Malaria Testing of Blood Unit by Rapid Method**

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| **SOP no.**  | **Effective Date** | **Pages** | **Prepared by**  | **Authorised by**  |
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| **LOCATION** : TTI Testing Laboratory |
| **SUBJECT** : Malaria Testing |
| **FUNCTION** : Samples tested for Malaria Antigen by rapid method |
| **DISTRIBUTION**: Supervisor in charge of TTI testing laboratory Master File |

1. **SCOPE & APPLICATION:**

Malaria is a serious, sometimes fatal, parasitic disease characterized by fever, chills, and anaemia 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 parasite that can infect human: Plasmodium falciparum, P. vivax, P. ovale, and P. malariae. 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 present, malaria is diagnosed microscopically by looking for the parasites in a drop of blood. ADVANTAGE MALARIA CARD is a visual, rapid and sensitive immunoassay for the qualitative differential detection of P. falciparum and P. Vivax malaria antigen in Human Blood only.

1. **RESPONSIBILITY:**

It is the responsibility of technician from TTI Testing lab to carry out the test and report as required.

1. **Material Required:**
	1. Disposable gloves
	2. Kit with test cards available
	3. Kit insert
	4. Blotting paper
	5. Paper napkin

***Specimen***

1. Collect the whole blood in a clean container (containing EDTA, citrate or heparin) by venipuncture. Fresh samples are preferred for testing as they perform best when tested immediately after collection. If samples are not immediately tested, they should be stored at 2-8oC for not more than 3 days, otherwise false / erroneous results may be obtained.
2. Haemolysed or clotted sample or sample with microbial contamination should not be used.
3. **PROCEDURE:**

**Principle:** Utilizes the principle of immunochromatography. As the test sample flows through the membrane assembly of the device after addition of the clearing buffer, the colored colloidal gold conjugates of monoclonal anti-Pf. HRP-2 (lgG) antibody and monoclonal anti Pan specific pLDH antibody complexes the HRP-2 / pLDH in the lysed sample. This complex moves further on the membrane to the test region where it is immobilized by the anti vivax specific pLDH (monoclonal) antibody and /or the monoclonal anti-Pf. HRP-2 (lgM) antibody coated on the membrane leading to
formation of pink-purple colored band/s which confirms a positive test result. A band will appear under Pf at the test region in falciparum positive samples, while a band will appear under Pv in vivax malaria positive samples. Appearance of band under Pf as well as Pv in the test region suggests a mixed infection. Absence of colored band/s in the test region indicates a negative test result. The unreacted conjugate and unbound complex if any, move further on the membrane and are subsequently immobilized by anti rabbit antibodies coated on the membrane at the control region, forming a pink-purple band. The control band formation is based on the 'Rabbit /anti-Rabbit globulin' system. Since it is completely independent of the analyte detection system, it facilitates formation of consistent control band signal independent of the analyte concentration. This control band serves to validate the test performance.

**SPECIMEN COLLECTION AND PREPARATION**

Fresh blood from finger prick *I* puncture should be used as a test specimen. However, fresh anti coagulated whole blood may also be used as a test sample and EDTA or Heparin or Oxalate can be used as suitable anticoagulant. The specimen should be collecled in a clean glass or plastic container. If immediate testing is not possible then the specimen may be stored at 2-8'C for upto 72 hours before testing. Clotted or contaminated blood samples should not be used for performing the test.

**Test Procedure and Interpretation of result**

* 1. Bring the complete kit and specimen to be tested to room temperature prior to testing.
	2. Remove the test card from the foil pouch prior to use. The test should be performed immediately after removing the test card from the foil pouch.
	3. Label the test card with donor’s number or identification number.
	4. Mix the anti-coagulated blood sample evenly by gentle swirling to make it homogeneous before use. Dip the sample loop into the sample and make sure that the loop is full of sample. Blot the blood onto the sample pad in the sample well ‘A’.
1. Place the nozzle on Assay Buffer vial as shown in BEFORE YOU START and add 5 drops of the the Assay buffer in the buffer well ‘B’.
2. Allow the reaction to occure for 20 minutes.
3. Read the result at 20 minutes.
4. Discard the card immediately after taking result at 20 minutes as it is potentially infectious.

**INTERPRETATION OF THE RESULTS**

**REACTIVE**

Appearance of three pink coloured line one each in P.v. region (V), P.f. region (F) & Control region (C) indicates that the sample is reactive for P. falciparum and P. vivax. As shown in Fig. (b) appearance of two pink coloured line one each at V & C region only indicates that the sample is reactive for P. vivax only. As shown in Fig. (c) appearance of two pink coloured line one each at F & C region only indicates that the sample is reactive for P. falciparum only. A difference of intensity in colour may occur between the test line & control line depending on the concentration of HRP-2 / pLDH in the sample but this does not affect the interpretation of the results. Depending on the concentration of pLDH/HRP-2, positive results may be observed within 60 seconds. However, to confirm a negative result the test result should be read only at 20 minutes. If the conc. of pLDH/HRP-2 in the sample is very high, only test line may be observed. This is due to Hook’s effect. Such samples should be diluted 1:10 or 1:20 in negative blood (Human) & again re-run the test, Diluted sample should show both control & test line. In case, if control line does not appear or is faint dilute the sample further.

**NON-REACTIVE**

Appearance of only one pink coloured line at Control(C) region indicates that the sample is non-reactive for P. vivax and P. falciparum.

**INVALID**

The test is invalid, if no line appears after the completion of test, either with clear background or with complete pinkish/ purplish background [Fig. (e)]. The test should be repeated using a new card.

* 1. **DOCUMENTATION:**

 **In the daily worksheet and rapid testing documentation its important to write;**

* 1. The date on which the test is run.
	2. The name of the kit used.
	3. Lot No. and expiry date of the kit.
	4. Initials of the technologist who performed the test.
	5. Initials of the Supervisor who verifies the result.
	6. Reactive units are marked in red.

Transfer the results to TTI register and in case of reactive samples immediately issue instructions or make sure personally to remove the unit along with the components prepared.

* 1. **REFERENCES:**
1. Technical Manual of the American Association of Blood Banks – 15th Edition, 2005.
2. Kit insert.

**7. END OF DOCUMENT**