



- Use protective clothing and wear gloves when handling samples.
- Use absorbent sheet to cover the working area.
- Immediately clean up any spills with Sodium hypochlorite.
- Dispose off all the reagents and material used as they contain infectious agent.
- Neutralize acid containing waste before adding hypochlorite.
- Sample diluent contains Sodium azide; avoid skin contact with this reagent. Azide may react with lead and copper in the plumbing and form highly explosive metal oxides. Flush with large volumes of water to prevent azide build-up in the plumbing .
- Do not use kit after the expiry date.
- Do not mix components of one kit with another.
- Always use new tip for each specimen and reagent.
- Do not allow liquid from one well to mix with other wells.
- Do not let the strips dry in between the steps.
- Mix blood sample before taking a specimen for testing.

#### A. STANDARD PROCEDURE (Preferred for automation)

##### REAGENT PREPARATION

- Dilute wash buffer 20 times (for example add 5 ml concentrated buffer to 95 ml distilled or deionized water).
- Dilute antibody reagent 50 times (for example add 20 µl reagent to 980 µl conjugate diluent).
- Dilute enzyme conjugate 50 times (for example add 20 µl concentrated enzyme conjugate to 980 µl conjugate diluent).

No. of strips	1	2	3	4	5	6	7	8	9	10	11	12
50X Antibody reagent (µl)	20	40	60	80	100	120	140	160	180	200	220	240
Conjugate Diluent (µl)	980	1960	2940	3920	4900	5880	6860	7840	8820	9800	10780	11760

No. of strips	1	2	3	4	5	6	7	8	9	10	11	12
50X Enzyme Conjugate (µl)	20	40	60	80	100	120	140	160	180	200	220	240
Conjugate Diluent (µl)	980	1960	2940	3920	4900	5880	6860	7840	8820	9800	10780	11760

##### TEST PROCEDURE

- Bring all the reagents and specimen to room temperature before use.
- Take out required number of strips and immediately close the pouch.
- Prepare data sheet indicating the location of controls and specimen.
- Use controls in duplicate.
- Add 25 µl control or whole blood specimen in separate wells, except A1 well.
- Add 100 µl Sample diluent in each wells except A1 well.
- Gently shake the plate to mix contents.
- Apply plate sealer and incubate for 30 minutes at 37°C.
- Wash each well by filling approximately 350 µl diluted wash buffer, giving 30 seconds soak time for each wash and aspirating/flicking off six times. Blot dry.
- Add 100 µl diluted antibody reagent in each wells, except A1 well & incubate at 37°C for 30 minutes.
- Wash six times as in step 9. Blot dry.
- Add 100 µl diluted conjugate in each well, except A1 well and incubate for 30 minutes at room temperature (22-28°C).
- Wash six times as in step 9. Blot dry.
- Add 100 µl substrate in each well, including A1 well and incubate at room temperature (22-28°C) away from light for 30 minutes.
- Stop reaction by adding 100 µl stop solution in each well, including A1 well. The stop solution should be added in the same sequence as substrate addition.
- Read the absorbance of each well at 450 nm with 600-700 nm as reference within 30 minutes of stopping the reaction.

#### B. RAPID PROCEDURE

##### REAGENT PREPARATION

- Dilute wash buffer 20 times (for example add 5 ml concentrated buffer to 95 ml distilled or deionized water).
- Dilute antibody reagent 50 times (for example add 20 µl reagent to 980 µl sample diluent).
- Dilute enzyme conjugate 50 times (for example add 20 µl concentrated enzyme conjugate to 980 µl conjugate diluent).

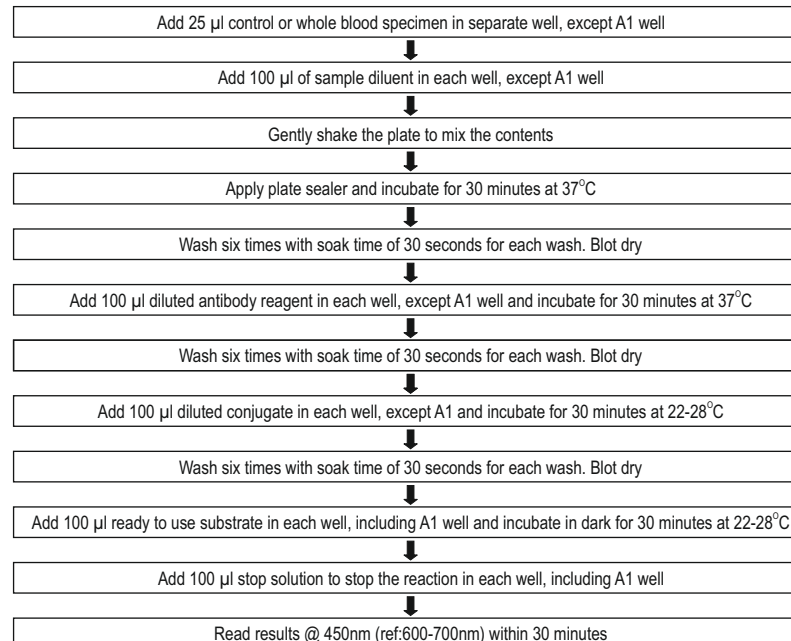
No. of strips	1	2	3	4	5	6	7	8	9	10	11	12
50X Antibody reagent (µl)	20	40	60	80	100	120	140	160	180	200	220	240
Sample Diluent (µl)	980	1960	2940	3920	4900	5880	6860	7840	8820	9800	10780	11760

No. of strips	1	2	3	4	5	6	7	8	9	10	11	12
50X Enzyme Conjugate (µl)	20	40	60	80	100	120	140	160	180	200	220	240
Conjugate Diluent (µl)	980	1960	2940	3920	4900	5880	6860	7840	8820	9800	10780	11760

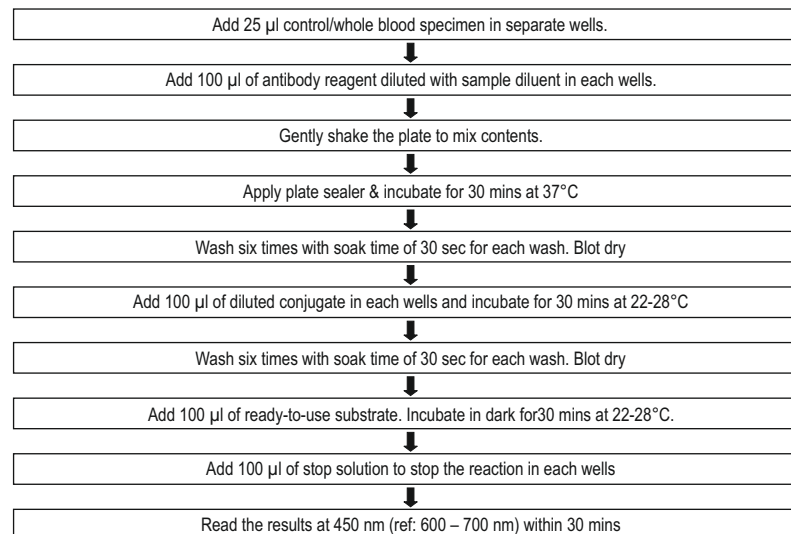
##### PROCEDURE

- Add 25 µl control or whole blood specimen in separate wells.
- Add 100 µl of antibody reagent diluted with sample diluent in each wells.
- Gently shake the plate to mix the contents. Incubate for 30 mins at 37°C.
- Wash six times with soak time of 30 sec for each wash. Blot dry.
- Add 100 µl of diluted conjugate in each wells and incubate for 30 mins at 22-28°C.
- Wash six times with soak time of 30 sec for each wash. Blot dry.
- Add 100 µl of ready-to-use substrate in each wells and incubate in dark for 30 mins at 22-28°C.
- Add 100 µl of stop solution to stop the reaction in each wells and read the results at 450 nm (ref: 600 – 700 nm) within 30 mins.

#### QUALISA™ MALARIA Procedure flow-chart A



#### QUALISA™ MALARIA Procedure flow-chart B



##### RUN CRITERIA

- The individual absorbance value of negative controls should be less than 0.1.
- The individual absorbance value of positive controls should be more than 1.0.
- If the test end does not meet above criteria, test run is invalid and should be repeated.

##### CALCULATIONS

In standard procedure, the absorbance of 'Blank Well' should be subtracted from the absorbance of test samples & controls. This is not applicable for rapid procedure.

The cut-off value (COV) is calculated by adding 0.1 to average absorbance value of negative control.