Example: **COV = Av.NC + 0.1**

Absorbance of Negative Control (NC)		Corrected Absorbance (Abs-blank)
Reading - 1	0.03	0.021
Reading - 2	0.027	0.018
Average NC reading		0.019

Absorbance of Positive Control (PC)		Corrected Absorbance (Abs-blank)
Reading - 1	2.823	2.814
Reading - 2	2.807	2.798
Average PC reading		2.806

SAMPLE DATA

Well	Absorbance	Corrected Absorbance (Abs-Blank)	Mean	Cutoff	Result
Blank*	0.009				
NC	0.03	0.021	0.019	0.119	
NC	0.027	0.018			
PC	2.823	2.814	2.806		
PC	2.807	2.798			
Sample 1	3.487	3.478			Reactive
Sample 2	0.654	0.645			Reactive
Sample 3	0.056	0.047			Non-Reactive

*Applicable for standard procedure and not applicable for rapid procedure.

INTERPRETATION OF RESULTS

(1) Samples with absorbance value less than the cutoff value are considered non-reactive by **QUALISA™ Malaria** ELISA kit and are considered negative for malaria. (2) Samples with absorbance value equal to or greater than cutoff value are considered reactive by **QUALISA™ Malaria** ELISA kit. The original sample should be retested in duplicate. Initially reactive sample that do not react in either of duplicate are considered negative for malaria. Initially reactive sample that reacts in either or both duplicates are considered repeatedly reactive. (3) If a sample is repeatedly reactive the probability of malaria infection are high, especially with patients at high risk or high absorbance values. Such samples should be retested with microscopy of thick smear and thin blood films. (4) In case of samples with high OD, there are possibilities of black precipitate formation after the addition of stop solution. This will not interfere with the interpretation of results.

PERFORMANCE CHARACTERISTICS

Note: Both the procedures will yield similar results.

In an in-house study, a panel of 284 samples whose results were confirmed with microscopy was tested with **QUALISA™ Malaria**.

The results are shown in the table below :

Samples	Total No. tested	QUALISA™ Malaria		Sensitivity	Specificity
		Positive	Negative		
P. falciparum	43	43	0	100%	
P. vivax	25	25	0	100%	
P. ovale	2	2	0	100%	
P. malariae	2	2	0	100%	
P. knowlesi	2	2	0	100%	
Malaria Negative	210	0	210		100%

LIMITATION OF THE PROCEDURE

QUALISA™ Malaria ELISA kit alone cannot be used to diagnose malaria infection even if the sample is repeatedly reactive or has high absorbance value. A physician can only establish clinical diagnosis. A negative result does not preclude the possibility of exposure to or infection with malaria.

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SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Batch Number / Lot Number	Date of Manufacture	Catalogue Number	*In vitro* Diagnostic Medical Device
Manufacturer	Do not reuse	Contains sufficient for <n> tests	This side up	Use by	Authorised Representative in the European Community

An ISO 13485 Certified Company

Manufactured by:

Qualpro Diagnostics

A Division of Tulip Diagnostics (P) Ltd.

88/89, Phase II C, Verna Industrial Estate, Verna, Goa - 403 722, INDIA.

Regd. Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex P.O., Goa - 403 202, INDIA.

EC REP

CMC Medical Devices & Drugs S.L.,
C/ Horacio Lengo No. 18,
CP 29006, Malaga, Spain.

0418/VER-03

QUALISA™ MALARIA

Enzyme Linked Immunosorbent Assay (ELISA) for the detection of Malaria specific antigen (pLDH) in human blood

Store at 2°C to 8°C

INTENDED USE

QUALISA™ Malaria is intended to be used for the qualitative detection of Malaria specific antigen (pLDH) in human whole blood samples. For In Vitro Diagnostic use only.

SUMMARY AND EXPLANATION

Five species of Plasmodium parasite are responsible for malaria infection in humans viz *P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi.* **QUALISA™ Malaria** detects the presence of malaria genus specific pLDH released by parasitised blood cells. Since pLDH is produced by viable parasites, the assay can also be used to monitor success of anti-malarial therapy. **QUALISA™ Malaria** is especially designed to exclude infected blood from the blood supply to prevent transfusion acquired malaria.

PRINCIPLE OF THE ASSAY

Agglutinating sera for Pan malaria specific pLDH is coated onto the wells of the microtiter strips. Samples are pipetted into the wells for binding to the Agglutinating sera for Pan malaria specific pLDH. After extensive washing to remove unbound material, pLDH is recognized by the addition of a biotinylated-Agglutinating sera for pan malaria specific pLDH. After removal of excess biotinylated - Agglutinating sera for pan malaria specific pLDH , streptavidin-peroxidase is added. Following a final washing, peroxidase activity is quantified using the substrate solution based on 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2). The intensity of the color reaction is measured at 450 nm after acidification and is directly proportional to the concentration of pLDH in the samples.

KIT COMPONENTS

QUALISA™ Malaria has following components

1. Coated microwells: Microwells are coated with Agglutinating sera for Pan malaria specific pLDH.
2. Positive Control: Agglutinating sera for mouse globulin with stabilizer. Produces a positive reaction.
3. Negative control: Bovine Serum Albumin with stabilizer. Produces a negative reaction and is used for cut-off calculations.
4. Enzyme Conjugate: Streptavidin-HRP conjugate (50 X). To be diluted 50 times with conjugate diluent.
5. Conjugate diluent: Buffered solution containing stabilizing proteins and preservatives.
6. Antibody Reagent: Agglutinating sera for Pan malaria specific pLDH (50 X) (follow reagent preparation protocol).
7. Sample diluent: Buffered solution containing stabilizing proteins and 0.1% sodium azide as preservative.
8. Substrate: Solution containing Tetramethyl benzidine (TMB) and hydrogen peroxide. Ready to use.
9. Wash Buffer: Buffer containing surfactants (20 X).To be diluted 20 times with distilled or deionized water.
10. Stop solution: Diluted Sulphuric acid (0.3N).
11. Microwell holder.
12. Instruction for use.
13. ELISA protocol sheet.
14. Plate sealer.

REF	405010096	405010192	405010480
Σ	96 Tests	192 Tests	480 Tests

STORAGE AND STABILITY

1. **QUALISA™ Malaria** kit is stable at 2-8°C upto the expiry date printed on the label.
2. Coated microwells should be used within one month of opening the pouch. Once opened, the pouch must be sealed properly to protect from moisture. If the colour of desiccant has changed from blue to white at the time of opening the pouch, another coated microwell pouch should be used.
3. Diluted conjugate must be used immediately.
4. Diluted wash buffer is stable upto one week.
5. Diluted antibody reagent should be used immediately.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Manual or automatic pipette.
2. Pipette tips.
3. Incubator.
4. Absorbent sheets.
5. ELISA washer.
6. ELISA reader.
7. Troughs or boats.
8. Disinfectant.
9. Reagent grade water.
10. Disposable gloves.
11. Timer.
12. Biohazard waste container.
13. Serological pipettes.

SAMPLE COLLECTION

1. No prior preparation of the patient is required.
2. Collect blood specimen by venipuncture according to the standard laboratory procedure.
3. Specimen should be free of particulate matter and microbial contamination.
4. Preferably use fresh whole blood sample. However, specimen can be stored refrigerated (2-8°C) up to three days. Specimen should be kept frozen at -20°C or lower, if sample storage is required for longer duration.
5. Specimens should be brought to room temperature prior to testing.
6. Anticoagulants (Heparin, EDTA or Citrate) don't interfere with the test results.

PRECAUTIONS

1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.

4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with Sodium hypoclorite.
7. Dispose off all the reagents and material used as they contain infectious agent.
8. Neutralize acid containing waste before adding hypoclorite.
9. 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 .
10. Do not use kit after the expiry date.
11. Do not mix components of one kit with another.
12. Always use new tip for each specimen and reagent.
13. Do not allow liquid from one well to mix with other wells.
14. Do not let the strips dry in between the steps.
15. Mix blood sample before taking a specimen for testing.

A. STANDARD PROCEDURE (Preferred for automation)

REAGENT PREPARATION

1. Dilute wash buffer 20 times (for example add 5 ml concentrated buffer to 95 ml distilled or deionized water).
2. Dilute antibody reagent 50 times (for example add 20 µl reagent to 980 µl conjugate diluent).
3. 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

1. Bring all the reagents and specimen to room temperature before use.
2. Take out required number of strips and immediately close the pouch.
3. Prepare data sheet indicating the location of controls and specimen.
4. Use controls in duplicate.
5. Add 25 µl control or whole blood specimen in separate wells, except A1 well.
6. Add 100 µl Sample diluent in each wells except A1 well.
7. Gently shake the plate to mix contents.
8. Apply plate sealer and incubate for 30 minutes at 37°C.
9. 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.
10. Add 100 µl diluted antibody reagent in each wells, except A1 well & incubate at 37°C for 30minutes.
11. Wash six times as in step 9.Blot dry.
12. Add 100 µl diluted conjugate in each well, except A1 well and incubate for 30 minutes at room temperature (22-28°C).
13. Wash six times as in step 9. Blot dry.
14. Add 100 µl substrate in each well, including A1 well and incubate at room temperature (22-28°C) away from light for 30 minutes.
15. 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.
16. 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

1. Dilute wash buffer 20 times (for example add 5 ml concentrated buffer to 95 ml distilled or deionized water).
2. Dilute antibody reagent 50 times (for example add 20 µl reagent to 980 µl sample diluent).
3. 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

1. Add 25 µl control or whole blood specimen in separate wells.
2. Add 100 µl of antibody reagent diluted with sample diluent in each wells.
3. Gently shake the plate to mix the contents. Incubate for 30 mins at 37°C.
4. Wash six times with soak time of 30 sec for each wash. Blot dry.
5. Add 100 µl of diluted conjugate in each wells and incubate for 30 mins at 22-28°C.
6. Wash six times with soak time of 30 sec for each wash. Blot dry.
7. Add 100 µl of ready-to-use substrate in each wells and incubate in dark for30 mins at 22-28°C.
8. 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

Add 25 µl control or whole blood specimen in separate well, except A1 well
↓
Add 100 µl of sample diluent in each well, except A1 well
↓
Gently shake the plate to mix the contents
↓
Apply plate sealer and incubate for 30 minutes at 37°C
↓
Wash six times with soak time of 30 seconds for each wash. Blot dry
↓
Add 100 µl diluted antibody reagent in each well, except A1 well and incubate for 30 minutes at 37°C
↓
Wash six times with soak time of 30 seconds for each wash. Blot dry
↓
Add 100 µl diluted conjugate in each well, except A1 and incubate for 30 minutes at 22-28°C
↓
Wash six times with soak time of 30 seconds for each wash. Blot dry
↓
Add 100 µl ready to use substrate in each well, including A1 well and incubate in dark for 30 minutes at 22-28°C
↓
Add 100 µl stop solution to stop the reaction in each well, including A1 well
↓
Read results @ 450nm (ref:600-700nm) within 30 minutes

QUALISA™ MALARIA Procedure flow-chart B

Add 25 µl control/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 contents.
↓
Apply plate sealer & 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. Incubate in dark for30 mins at 22-28°C.
↓
Add 100 µl of stop solution to stop the reaction in each wells
↓
Read the results at 450 nm (ref: 600 – 700 nm) within 30 mins

RUN CRITERIA

1. The individual absorbance value of negative controls should be less than 0.1.
2. The individual absorbance value of positive controls should be more than 1.0.
3. 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.