2. **ELECTRA™ MAL Ag CLIA** was also evaluated using 12 P. Falciparum positive and 02 P. Vivax positive samples against other Malaria EIA and confirmed with microscopy.
3. **ELECTRA™ MAL Ag CLIA** was evaluated using Paratrol Pf and Paratrol Pv for sensitivity using serial dilution against other Malaria EIA.
4. **ELECTRA™ MAL Ag CLIA** was evaluated using serial dilution of in house Malaria positive panel samples against other Malaria EIA.

REMARKS

1. **ELECTRA™ MAL Ag CLIA** kit alone cannot be used to diagnose malaria infection even if the sample is repeatedly reactive or as high RLU.
2. As with all immunoassays, the **ELECTRA™ MAL Ag CLIA** may yield non-specific reactions due to other causes, particularly when testing in low prevalence populations. A repeatedly reactive specimen should be tested further with supplemental confirmatory tests.
3. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
4. A negative result does not preclude the possibility of exposure to or infection with malaria.

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9. Data on file: Qualpro Diagnostics, A Division of Tulip Diagnostics (P) Ltd.

SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Date of Manufacture	LOT Batch Number / Lot Number
Manufacturer	IVD *In vitro* Diagnostic Medical Device	This side up	Σ Contains sufficient for <n> tests
Use by	REF Catalogue Number	Do not reuse	

Manufactured by:

Qualpro Diagnostics

A Division of Tulip Diagnostics (P) Ltd.

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An ISO 13485 Certified Company

1220/VER-03

electra™ MAL Ag

Chemiluminescence Assay for the detection of Malaria specific antigen (pLDH) in human blood.
FOR IN VITRO DIAGNOSTIC USE ONLY

INTENDED USE

ELECTRA™ MAL Ag CLIA is intended to be used for the qualitative detection of Malaria specific antigen (pLDH) in human whole blood samples.

SUMMARY

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

PRINCIPLE

The **ELECTRA™ MAL Ag CLIA** assay is for use on **ELECTRA™ SA** and **ELECTRA™ FA** analyzers. **ELECTRA™ MAL Ag CLIA** works on the principle of chemiluminescence wherein light is produced by a chemical reaction from a substance as it returns from an electronically excited state to the ground state. When catalysed by HRP, the oxidation of luminol by hydrogen peroxide produces an electronically excited form of 3-aminiphthalate which on relaxation emits light with maximum intensity at λ=425nm.

ELECTRA™ MAL Ag CLIA micro-well strips are coated with agglutinating sera for pan malaria specific pLDH. 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 serum for pan malaria specific pLDH. After removal of excess biotinylated - agglutinating sera for pan malaria specific pLDH, streptavidin-peroxidase is added. After washing wells to remove unbound enzyme, chemiluminescent substrate is added. The bound enzyme converts substrate to a reaction product that emits a photon of light.

Chemiluminescence is measured in Relative Light Units (RLU) that is typically proportionate to the amount of analyte present in the sample. The presence or absence of analyte in the sample is determined by comparing the sample RLU with Cut-off which is calculated by **ELECTRA™ SA** and **ELECTRA™ FA** analyzers and expressed as Electra cutoff Index (ECI). ECI is equivalent to S/CO ratio which is calculated by using sample RLU & calculated Cutoff of the specific testing batch. Samples with ECI values greater than or equal to 1.00 are considered reactive and samples with ECI values less than 1.00 are considered nonreactive.

KIT COMPONENTS

ELECTRA™ MAL Ag CLIA has following components:

1. Coated micro-wells: Micro-wells (3 x 8 wells) are coated with Agglutinating sera for Pan malaria specific pLDH. Ready to use.
   96 Wells: (3x8) x 4 pouches, 192 Wells: (3x8) x 8 pouches.
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 & is used for cut-off calculation.
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 (50X) (follow reagent preparation protocol).
7. Sample Diluent: Buffered solution containing stabilizing protein & 1% sodium azide as preservative.
8. Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
9. Substrate B: Chemiluminescent substrate containing stabilized peroxide solution.
10. Wash buffer: Buffer containing surfactants (20 X). To be diluted 20 times with distilled or deionized water.
11. Microwell holder.
12. Instruction for use.
13. Plate sealer.
14. CLIA protocol sheet.

REF	407030096	407030192
Σ	96 Tests	192 Tests

STORAGE AND STABILITY

1. **ELECTRA™ MAL Ag CLIA** kit is stable at 2-8°C up to the expiry date printed on the label.
2. Coated micro-wells should be used within one month of opening the pouch. Once opened, the pouch must be sealed properly to protect from moisture. In case the desiccant pouch changes color from blue to white, the strips should not be used.
3. Diluted conjugate must be used immediately.
4. Diluted wash buffer is stable up to one week at 2-8°C.
5. Working Substrate (A+B) must be used immediately.
6. Diluted antibody reagent must be used immediately.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Manual or automatic pipettor.
2. Pipettor tips.
3. Incubator.
4. Micro-well washer
5. **ELECTRA™ SA** or **ELECTRA™ FA**.
6. Reagent grade water.
7. Disposable gloves
8. Timer/ Stop Watch.

SAMPLE COLLECTION

1. No prior preparation of the patient is required.
2. Collect blood specimen by venipuncture according to the standard 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 for upto three days. However for long storage, samples should be frozen at -2°C or below.
5. Specimen should be brought to room temperature prior to testing.
6. Anticoagulants (Heparin, EDTA or Citrate) don't interfere with the test results.

PRECAUTIONS

1. Do not pipette any material by mouth.
2. Do not eat, drink or smoke in the area where testing is done.
3. Use protective clothing and wear gloves when handling samples.
4. Immediately clean up any spills with sodium hypochlorite.
5. Dispose off all the reagents and material used as if they contain infectious agent.
6. Neutralize acid containing waste before adding hypochlorite.
7. Do not use the kit after the expiry date.
8. Do not mix the components of one kit with those from another.
9. Always use a new tip for each specimen and reagent.
10. Do not allow liquid from one well to mix with other wells.
11. Do not let the strips dry in between the steps.
12. Do not expose working substrate to direct light.

REAGENT PREPARATION

1. Mix the Antibody Reagent & Sample Diluent according to requirement as shown below:

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
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

2. Dilute wash buffer 20 times (for example add 5 ml concentrated buffer to 95 ml distilled or deionized water).
3. Dilute enzyme conjugate with Conjugate diluent according to the requirement as shown below. Prepare a fresh dilution for each assay.

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
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

4. Prepare a Working Substrate by Mixing Substrate A and Substrate B in equal volume (1:1 ratio) before addition to the micro-wells.

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
Substrate – A (µl)	250	450	650	850	1050	1250	1450	1650	1850	2050	2250	2450
Substrate – B (µl)	250	450	650	850	1050	1250	1450	1650	1850	2050	2250	2450

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. Well A1 must be used as blank.
4. Add 25 µl of NC's in wells B1 & C1 & PC's in wells D1 & E1 respectively.
5. Add 25 µl of samples to the remaining wells.
6. Add 100 µl Diluted Antibody Reagent in each well except well A1.
7. Apply plate sealer and incubate for 15 minutes at 37°C.
8. Wash each well using a semi-automated micro-plate washer (Preferably Lisa Wash models) with diluted wash buffer for 6 wash cycles giving one minute soak time for each wash cycle and Blot dry.
9. Add 100 µl Diluted Conjugate in each well except well A1 and incubate for 15 minutes at 18-28°C.
10. Wash six times as described in step 8 and Blot dry.
11. Add 50 µl of working Substrate (A+B) in all the micro-wells. Keep away from direct light while adding the substrate.
12. Cover the Electra microplate and incubate for 10 mins at room temperature (18-25°C) in dark.
13. Read the Electra micro-plate exactly at 10 mins in **ELECTRA™ SA** or **ELECTRA™ FA.** If Electra micro-plate is not read between 10- 15 mins, the test results should be considered as invalid.

TEST VALIDATION CRITERIA

1. The individual RLU of negative controls should be less than 200000 for **ELECTRA™ SA** and **ELECTRA™ FA.**
2. The individual RLU of positive controls should be more than 7500000 for **ELECTRA™ SA** and **ELECTRA™ FA.**

CALCULATIONS

The cutoff value is calculated by the software of **ELECTRA™ SA** and **ELECTRA™ FA** using the mean of negative control RLU and a factor.
Factor for **ELECTRA™ SA** and **ELECTRA™ FA** is 500000

SAMPLE DATA

For **ELECTRA™ SA** and **ELECTRA™ FA:**
Considering the Cutoff for this batch is **508853**

Well	RLU	ECI	Interpretation
Blank	53		
NC	8140		
NC	9566		
PC	13394488		
PC	13573076		
sample 1	5550037	10.9	Reactive
sample 2	140816	0.27	Non-reactive
sample 3	1020724	2.01	Reactive
sample 4	8388	0.02	Non-reactive

INTERPRETATION OF RESULTS

1. Results are interpreted in E.C.I. This is determined by dividing the RLU of the sample by the Cutoff value calculated for that specific run.
2. Samples with E.C.I values greater than or equal to 1.00 are considered Reactive and samples with E.C.I values less than 1.00 are considered Non-reactive.
3. Samples that are initially reactive in **ELECTRA™ MAL Ag CLIA** should be retested in duplicate. Repeated reactivity is highly predictive Malaria infection.
4. A grey zone of ± 10% is recommended.

PERFORMANCE CHARACTERISTICS

1. **ELECTRA™ MAL Ag CLIA** was evaluated with 301 random samples out of which 300 were negative samples and 01 was positive. The results were compared with commercially available Malaria EIA.

Specimen Data	Total	ELECTRA™ MAL Ag CLIA	Other Malaria EIA
Total Specimens	301	301	301
Malaria Reactive	01	01	01
Malaria Non-Reactive	300	300	300