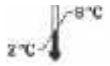
MALARIA ANTIGEN ELISA

REF 650101 IVD



1. INTENDED USE

The **apDia Malaria Antigen ELISA** is an *in vitro* diagnostic immunoassay (IVD) for the qualitative determination of *Plasmodium* spp LDH in blood samples. The apDia Malaria antigen test can be used for the detection of the malarial antigen pLDH of any of the four species in blood samples. It may also be used for culture samples to measure *in vitro* drug susceptibility.

2. BACKGROUND

Malaria is a serious, sometimes fatal blood-born parasitic disease resulting from infection with protozoa of the genus *Plasmodium* and is transmitted by the bite of *Anopheles* mosquitoes. About half of the world's population is at risk of malaria, leading to 250 million malaria cases yearly and nearly one million deaths. Four species infect humans: *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*.

Prompt and accurate diagnosis of malaria is needed for implementation of appropriate treatment to reduce associated morbidity and mortality. The first symptoms (most often fever, chills, sweats, headaches, muscle pains, nausea and vomiting) are not specific and may resemble those of many other infectious diseases like the flu. In severe malaria, clinical findings (confusion, coma, neurologic focal signs, severe anemia, respiratory difficulties) are more striking and may increase the suspicion index for malaria. It is essential to confirm the clinical diagnosis with results from the laboratory. Treatment must start early to cure the disease effectively and is also important in preventing the spreading of the disease. Laboratory diagnosis of malaria is based on the identification of the malaria parasite or its antigens in the blood of the patient. While microscopy is considered as the gold standard, assays detecting the *Plasmodium* specific enzyme lactate dehydrogenase (pLDH) are considered a prominent alternative. All four human malarial parasites produce a unique pLDH activity and its presence follows the level of parasitemia making it a good monitoring tool for following active malarial infections. Even though malaria is a tropical disease, cases of malaria may appear all over the world due to increased travelling practices. As the disease may also be transmitted by blood transfusion, control of donor blood for the presence of the parasite is essential.

3. PRINCIPLE OF THE TEST

The apDia Malaria antigen test is a sandwich Enzyme-Linked Immuno-Sorbent Assay (ELISA) for the detection of the malarial antigen pLDH of any of the four species in blood samples. pLDH is present in parasite infected erythrocytes.

A monoclonal antibody pan-specific for all species of pLDH is coated onto the wells of the microtiter strips. Samples are pipetted into the wells for binding to the immobilized antibody. After extensive washing to remove unbound material, pLDH is recognized by the addition of a biotinylated monoclonal antibody also pan-specific for pLDH. After removal of excess biotinylated antibody, 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.

4. KIT COMPONENTS

1. Coated Microtiterstrips - MTP
2 x 96 well microplates (24 breakable 8-well-strips) coated with mouse monoclonal antibodies to pLDH.

2. Negative Control - CTLNEG
1 x 1.8 mL negative control; generates O.D. values comparable to fresh negative blood samples; ready-to-use green solution.

3. Positive Control - CTLPOS
2 x 0.5 mL lyophilized pLDH in protein matrix; contains approximately 10ng recombinant pLDH. To be reconstituted with 0.5 mL lysing buffer (see "Reconstitution of Reagents").

4. Lysing Buffer - LYS
1 x 30 mL lysing buffer with ammonium chloride as lysing agent, ready-to-use.

5. Conjugate Solution 1 - CONJ1
1 x 25 mL of biotinylated monoclonal anti-pLDH antibody in a ready-to-use blue solution.

6. Conjugate Solution 2 – CONJ2
1 x 25 mL horseradish peroxidase conjugated streptavidin in a ready-to-use red solution.

7. Washing Solution - WASH 20x
2 x 50 mL (20x) concentrated washing solution containing detergent and preservatives.

8. Chromogen Solution - CHROM
2 x 13 mL chromogen/substrate solution, ready-to-use.

9. Stopping Solution - STOP
1 x 15 mL diluted sulphuric acid (0.5M), ready-to-use.

5. MATERIALS REQUIRED BUT NOT SUPPLIED

- Micropipettes with disposable tips
- Clean standard laboratory volumetric glassware
- Microplate incubator-agitator
- ELISA plate reader with 450 and 620-630 nm filters

6. WARNINGS AND PRECAUTIONS FOR USERS

- For *in vitro* diagnostic use only.
- Dispose patient samples and all materials used to perform this test as if they contain infectious agents.
- Do not mix reagents or coated microtiterstrips from kits with different lot numbers.
- Some kit components contain sodium azide as a preservative. In order to prevent the formation of potentially explosive metal azides in laboratory plumbing, flush drains thoroughly after disposal of these solutions.

7. STORAGE CONDITIONS

- Store the microtiterstrips in their original packaging, sealed, with the desiccant until all the strips have been used.
- Never use any kit component beyond the expiration date.
- Do not expose chromogen solution (**8**) to strong light or high temperature during storage. This solution should be colourless; if not, it should be replaced.
- Opened components should be stored at 2 – 8 °C until next use and can be maintained for at least one year.
- For storage of reconstituted components, see chapter 9.2 "Reconstitution of Reagents"

8. SPECIMEN COLLECTION AND HANDLING

Draw blood samples using acceptable phlebotomy techniques. Blood samples can be drawn into Citrate, EDTA, Heparin or CPD-A tubes. Only fresh whole blood samples should be used. If the blood sample cannot be tested immediately, it should be refrigerated at 2-8 °C and can be maintained for up to one week.

9. ASSAY PROCEDURE

9.1 General remarks

- Use a separate disposable tip for each sample transfer to avoid cross-contamination.
- All reagents must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the assay has been started, all steps should be completed without interruption.
- If a dilution of a positive sample has to be made, it should preferably be prepared by using fresh negative blood as diluent.

9.2 Reconstitution of Reagents:

- *Washing Solution*: dilute 50 mL of concentrated washing solution (**7**) to 1000 mL with distilled water. Reconstituted solution can be stored for at least 1 month at 2 – 8 °C.
  (At elevated temperatures, the concentrated washing solution (**7**) may appear cloudy without affecting its performance. Upon dilution, the solution will be clear.)
- *Positive Control:* Reconstitute the contents of the positive control vial **(3)** with 0.5 mL of lysing buffer. Reconstituted controls may be kept at 2-8 °C for up to two weeks. For long term storage, aliquots may be stored at -20 °C. Once thawed, do no refreeze.

9.3 Use of ELISA washing device

It might be necessary to adapt the washing procedure depending on the automatic microplate washer used. If necessary, increase the number of cycles and include a soak time after each wash cycle based on the OD values of the negative and positive control.

9.4 Assay Procedure

- Put the needed strips/wells **(1)** in the microplate frame (incl. 4 wells for controls). Place unused wells in the aluminium bag, reseal and store at 2–8 °C.
- Dispense 100 µL of lysing buffer **(4)** into each well.
- Add 50 µL of reconstituted positive control to one well and 50 µL of negative control **(2)** in triplicate wells.
- Add 50 µL of homogenized fresh whole blood samples into the corresponding wells (it is recommended to dispense the sample immediately after mixing it).
- Cover the wells with self-adhesive plate cover foil. Incubate for 60 minutes at 37 °C under continuous gentle shaking conditions.
- Empty the wells entirely by aspiration. Fill the wells completely with 350-400 µL of reconstituted washing solution, avoiding overflow of buffer from one well to another. Allow the wells to soak for 1 minute. Repeat the wash and soak procedure four more times for a total of five washes. Finally, aspirate the content of the wells and remove any residual liquid by gently tapping the inverted wells on clean absorbent paper. Incomplete washing will adversely affect the test outcome.

- Dispense 100 µL of conjugate solution 1 **(5)** into each well.
- Cover the plate and incubate for 30 minutes at 37 °C (shaking is not necessary).
- Wash the wells 5 times with reconstituted washing solution as described above.
- Dispense 100 µL of conjugate solution 2 **(6)** into each well.
- Cover the plate and incubate for 15 minutes at 37 °C (shaking is not necessary).
- Wash the wells 5 times with reconstituted washing solution as described above.
- Dispense 100 µL of chromogen solution **(8)** into each well.
- Cover the plate and incubate for 15 minutes at 37 °C. Avoid light exposure during this step (shaking is not necessary).
- Add 50 µL of stopping solution **(9)** into all wells and read absorbance of each well with ELISA spectrophotometer at 450 nm with reference wavelength at 620 or 630 nm within 15 minutes.
- Record the results.

10. CALCULATION OF RESULTS

10.1 Test validity

- The individual absorbance value (optical density OD) of the positive control (ODpos) must be above 0.500.
- The average OD of the negative control (ODneg) must be lower than 0.100.
- If one of these specifications is not met, the results should be considered invalid and the series of tests should be repeated.

10.2 Cut-off calculation and calculation of Ag Index

- ODneg is used to calculate the cut-off by multiplying its value by 3:

Cut-off value = ODneg x 3

- The antigen index (Ag Index) of each sample is calculated by dividing the OD value of the sample (ODsample) by the cut-off value:

Ag Index = ODsample / Cut-off value

10.3 Interpretation of the results for detection of *Plasmodium* spp in whole blood specimens

A positive reaction corresponds to an Ag Index above or equal to 1.0
A negative reaction corresponds to an Ag Index below or equal to 0.8.
A grey-zone of Ag Index between 0.8 and 1.0 has to be considered because some samples can produce higher backgrounds than others. This can mimic or mask low reactivity. Samples inside this "grey-zone" should be considered as doubtful and should be retested, preferably using a freshly drawn blood sample in order to assess a possible recent malaria infection.

Negative result: Ag Index ≤ 0.8
Positive result: Ag Index ≥ 1.0
Doubtful result: 0.8 < Ag Index < 1.0

For parasite detection in fresh whole blood samples, a positive result indicates that live malaria parasites are detected. However, no distinction between *P. falciparum, P. vivax, P. malariae or P. ovale* is possible.
A negative result indicates the absence of detectable live human plasmodia in the sample tested.

The result must be interpreted within the epidemiological, clinical and therapeutic context.

11. LIMITATIONS OF PROCEDURE

- Contamination of the materials used can cause aberrant results.
- Old or frozen-thawed whole blood samples may lead to erroneous results and should not be used.
- Strict adherence to the protocol is necessary.

12. PERFORMANCE

12.1 Analytical Sensitivity - *Plasmodium falciparum* detectability

Plasmodium falciparum recombinant pLDH dilutions in blood have shown a detection limit below 0.2 ng/mL. The detection limit of *P. falciparum* is below 1 parasite/µL (strain 3D7).

12.2 Diagnostic Sensitivity

The evaluation of a panel of malaria infected patients gave a sensitivity of 100% for *P. falciparum* (63/63), for *P. malariae* (1/1) and for *P. vivax* (2/2). For *P. ovale*, 3/6 positive samples were detected. The detection is good for samples from patients under treatment: pLDH was still detectable 8 days after anti-*Plasmodium* treatment (8/8).

12.3 Specificity

Specificity on 48 fresh blood samples from healthy donors was 100%.

12.4 Precision

Based on the OD values obtained from intra-assay runs, the precision of the assay can be described for in house controls as follows:

Mean values (n = 30)	*pLDH control, level 1*	*pLDH control, level 2*
OD 450 nm	*0.462*	*1.510*
% CV	*6.9*	*6.1*

Based on the Ag Index values obtained from inter-assay runs, the precision of the assay can be described for pLDH positive samples as follows:

Mean values (n = 12)	*pLDH positive, level 1*	*pLDH positive, level 2*
Ag Index, mean value	*5.8*	*41.6*
% CV	*12.7*	*10.1*

13. QUALITY CONTROL

The use of a positive and negative control allows easy validation of kit stability. For a valid test, the positive control must have an OD value of at least 0.5 and the mean OD value of the negative controls must be less than 0.1. Should one of the values fall outside these limits, the test should be repeated.

14. TROUBLE SHOOTING

Problem: Negative control has substantial color development.
Correction: Washings were insufficient. Repeat test with more vigorous washings.

BIBLIOGRAPHY

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apDia

In vitro diagnostic kit

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