



Unimed Product Line – Malaria Rapid Diagnostic Tests (RDT): Pros and Cons

The World Health Organization estimates that 300-500 million cases of malaria occur and 2 – 3 million people die of malaria yearly. Approximately 90% of these deaths occur in Africa and most occur in children under five years of age.

Malaria in humans is caused by four species of Plasmodium parasites: *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. The tropical zone is the endemic home of all parasites. *Plasmodium falciparum* is the most common and fatal species in tropical areas. *P. malariae* is a parasite of sub-tropical zone. *P. vivax* is the prevalent species in the temperate zone. The distribution of *P. ovale* has been reported mainly from East Africa, West Africa and from the Philippines in Asia.

Out of total occurrence of malaria cases globally, it is estimated that about 40% are caused by *P. falciparum*; about 50% caused by *P. vivax*, about 7 – 8 % caused by *P. malariae* and rest by *P. ovale*.

FIRST GENERATION MALARIA RAPID DIAGNOSTIC TESTS

FirstSign™ – Malaria Pf

A rapid Immunochromatographic test for the qualitative detection of Plasmodium falciparum (P. f.) specific histidine rich protein-2 (Pf HRP-2) in human Whole blood.

PROS

- Sensitivity and specificity >98% for *P. falciparum* malaria.
- Results comparable with Gold Standard Tests (Microscopy & PCR).
- No cross reactivity with other Plasmodium species.
- Correlates with onset of clinical symptoms.

CONS

- Stand-alone test for only one malaria species: *P. falciparum*.
- Cannot be used for Drug monitoring therapy as HRP-2 levels persist up to about 15 days post treatment.
- Cannot identify “Carriers” due to absence of HRP-2 in gametocytes.

FirstSign™ – ParaView (Pan + Pf)

A rapid Immunochromatographic test for the qualitative detection of and Pan specific pLDH (All four Malaria species) and Plasmodium falciparum (P. f.) specific histidine rich protein-2 (Pf HRP-2) in human Whole blood.

PROS

- Pan Malaria band is specific for all 4 malarial species.
- High sensitivity for *P. falciparum*.

CONS

- Differentiation between stand-alone *P. falciparum* and mixed infections is not possible.
- In endemic areas of high prevalence of mixed infection, need for specific detection and differentiation under microscopy for correct therapy.
- Presumption of causative species based on the presence of Pan Malaria band and species prevalence rate.

The two major Rapid Malaria Tests available in the market: **OpitIMAL** (Pf pLDH + Pan pLDH combination) and **NOW ICT** (Pf HRP-2 + Pan Aldolase combination) are First Generation Tests, and have the same limitations like our **FirstSign™ – ParaView (Pan + Pf)** test.

SECOND GENERATION MALARIA RAPID DIAGNOSTIC TESTS

Unimed group has introduced the **World's first** Innovative and state-of-the-art **Second Generation** RDTs for the Specific Detection and Differentiation of Malaria and follow up for the efficacy of ant-malaria therapy.

FirstSign™ – ParaView – 2 (Pv + Pf)

A rapid Immunochromatographic test for the qualitative detection and differentiation of Plasmodium vivax (Pv) and Plasmodium falciparum (Pf) in human Whole blood.

PROS

- Unique Rapid test for Specific Detection and Differential Diagnosis of *P. vivax* and *P. falciparum* Malaria as the test detects the presence of *P. vivax* specific pLDH and *P. falciparum* specific pLDH.
- Ideal screening aid at the Primary Care level and Field-testing of Malaria with remote testing facilities.
- Test and Treat in one visit.
- Ideal test for monitoring Anti-malarial therapy.

CONS

- Cannot detect *P. malariae* and *P. ovale*.

FirstSign™ – ParaView – 3 (Pan + Pv + Pf)

A rapid Immunochromatographic test for the qualitative detection Pan malaria specific pLDH, P.vivax specific pLDH and P.falciparum specific histidine rich protein-2 (Pf HRP-2) in human Whole blood.

PROS

- Can specifically detect and differentiate between the “Big Tow Malaria – Pf & Pv”, which constitute about 90% of the total malaria occurrence globally.
- Good clinical sensitivity and specificity >98% for *P. falciparum* malaria, due to HRP-2 based detection.
- Able to monitor success of anti-malarial therapy, through pLDH band.
- Overcomes limitations associated with stand-alone HRP-2 based tests.

CONS

- Cannot specifically detect and differentiate *P. malariae* and *P. ovale*.

FirstSign™ – PanCheck (Pv + Pf + Pm + Po)

Diagnostic Designed to prevent Transfusion Transmitted Malaria.

A rapid Immunochromatographic test for the qualitative detection Pan malaria specific pLDH (P. falciparum, P.vivax, P.ovale and P.malariae) in human Whole blood.

PROS

- Ensures detection of all four malaria specific Plasmodium species (*P. falciparum*, *P.vivax*, *P.ovale* and *P.malariae*).
- Detects the viable parasites, thus identifying only the current malaria infection.
- Ideal test for screening potential blood donors.
- Useful for screening collected blood bags for malaria.
- Able to monitor success of anti-malarial therapy.

CONS

- Cannot specifically detect and differentiate *P. falciparum*, *P.vivax*, *P.ovale* and *P.malariae*.



Rapid Test for Malaria – Pf + Pan

A rapid Immunochromatographic test for the qualitative detection of Plasmodium falciparum (P. f.) specific histidine rich protein-2 (Pf HRP-2) and Pan specific pLDH in Whole blood

INTRODUCTION

Unimed **FirstSign™ – ParaView** is a rapid self-performing, qualitative, two site sandwich immunoassay, utilizing whole blood for the detection of *P.falciparum* specific histidine rich protein-2 (Pf HRP-2) and Pan specific pLDH. The test may also be used for differentiation of *P.falciparum* and other malarial species and for the follow up of antimalarial therapy, in whole blood samples.

SUMMARY

Four species of the Plasmodium parasites are responsible for malaria infections in human viz. *P.falciparum*, *P.vivax*, *P.ovale* and *P.malariae*. Of these *P.falciparum* and *P.vivax* are the most prevalent. Early detection and differentiation of malaria is of paramount importance due to incidence of cerebral malaria and drug resistance associated with falciparum malaria and due to the morbidity associated with the other malarial forms.

FirstSign™ – ParaView detects the presence of pan malaria specific pLDH released from the parasitised blood cells, for the detection of all malarial parasites. Whereas, for the detection of *P.falciparum* malaria, **FirstSign™ – ParaView** utilizes the detection of *P.falciparum* specific histidine rich protein-2 (Pf HRP-2), which is a water soluble protein that is released from parasitised erythrocytes of infected individuals.

In the absence of *P.falciparum* specific Pf HRP-2, the presence of the pan malaria specific band points to the presence of other malarial species; viz.; *P.vivax*, *P.ovale* or *P.malariae*. Speciation is done and results inferred in the context of prevalence rates of the malarial species prevalent in the particular region.

Since pLDH is a product of viable parasites, the pan band may also be used to monitor success of antimalarial therapy.

PRINCIPLE

FirstSign™ – ParaView 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 monoclonal anti HRP-2 specific / anti pan specific -colloidal gold conjugate antibodies complexes the proteins in the lysed sample. This complex moves further on the membrane to the test region where it is immobilized by the monoclonal anti HRP-2 / anti pan specific antibody coated on the membrane leading to formation of purple colored band/s which confirms a positive test result. While both the bands will appear at the test region in falciparum positive samples, only one band would appear in non-falciparum malaria positive samples. Absence of this colored band/s in the test region indicates a negative test result.

The unreacted conjugate along with the rabbit globulin colloidal gold 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. This control band serves to validate the test performance.

REAGENTS AND MATERIAL SUPPLIED

FirstSign™ – ParaView kit contains:

A. Individual pouches, each containing:

1. Test Device: Membrane assembly predisposed with monoclonal anti HRP-2 -colloidal gold conjugate, monoclonal anti pan specific pLDH -colloidal gold conjugate, rabbit globulin–colloidal gold conjugate and monoclonal anti HRP-2 antibody, monoclonal anti pan specific pLDH antibody and anti-rabbit antibody at the respective regions.
2. Desiccant pouch.
3. 5 µl sample loop.
- B. Clearing buffer in a dropper bottle.
- C. Package insert.

OPTIONAL MATERIAL REQUIRED

Calibrated micro pipettes capable of delivering 5 ml sample accurately.

STORAGE AND STABILITY

The test kit may be stored between 4-30°C till the duration of the shelf life as indicated on the pouch / carton. DO NOT FREEZE.

NOTE

Read the instructions carefully before performing the test. For in vitro diagnostic use only. NOT FOR MEDICINAL USE. Do not use beyond expiry date. Do not inter mix reagents from different lots. Handle all specimens as potentially infectious. Follow standard biosafety guidelines for handling and disposal of potentially infective material.

SPECIMEN COLLECTION AND PREPARATION

Fresh anti coagulated whole blood should be used as a test sample and EDTA or Heparin or Oxalate can be used as suitable anticoagulant. The specimen should be collected in a clean glass or plastic container. If immediate testing is not possible then the specimen may be stored at 2–8°C for up to 72 hours before testing. Clotted or contaminated blood samples should not be used for performing the test. Fresh blood from finger prick / puncture may also be used as a test specimen.

TEST PROCEDURE

1. Bring the **FirstSign™ – ParaView** kit components to room temperature before testing.
2. Open the pouch and retrieve the device, sample loop and desiccant. Check the color of the desiccant. It should be blue. If it has turned colorless or pink, discard the device and use another device. *Once opened, the device must be used immediately.*
3. Tighten the vial cap of the clearing buffer provided with the kit in the clockwise direction to pierce the dropper bottle nozzle.
4. Evenly mix the anti coagulated blood sample by gentle swirling. Dip the sample loop into the sample. Ensuring that a loop full of blood is retrieved, blot the blood so collected on to the sample pad in the sample port 'S'. (This delivers approximately 5 µl of the whole blood specimen)

OR

In case finger prick blood is being used, touch the sample loop to the blood on the finger prick. Ensuring that a loop full of blood is retrieved, immediately blot the specimen on to the sample pad in the sample port 'S' (Care should be taken that the blood sample has not clotted and the transfer to the sample pad is immediate).

OR

Alternatively, 5 µl of the anticoagulated or finger prick specimen may be delivered to the sample pad in the sample port 'S' using a micropipette.

NOTE: Ensure the blood from the sample applicator pipette has been completely taken up by the sample pad.

5. Dispense six drops of the clearing buffer into port 'R', by holding the plastic dropper bottle vertically.
6. At the end of 15 minutes read the results as follows:

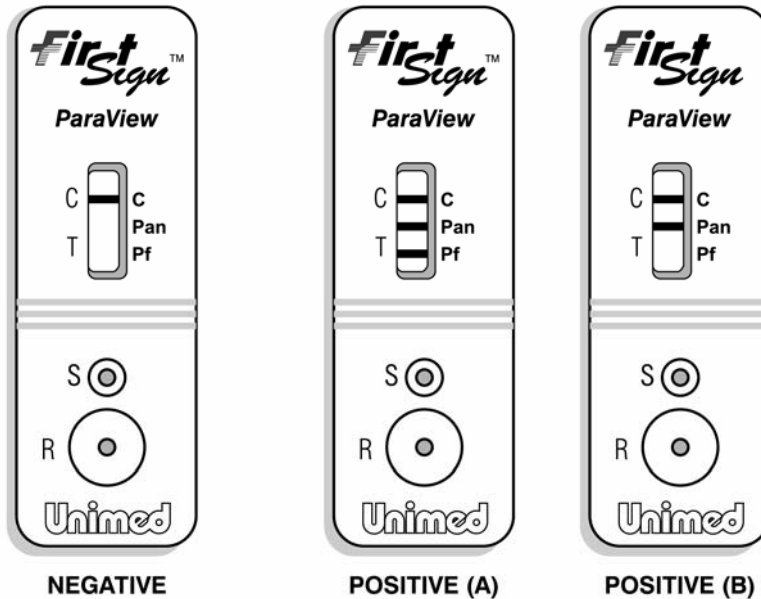
NEGATIVE for malaria:

Only one pink / pink-purple band appears in the control window 'C'.

POSITIVE for malaria:

POSITIVE (A): *P. falciparum* or mixed infection: In addition to the control band, two pink / pink-purple bands appear at regions 'Pf' and 'Pan' in the test window 'T'.

POSITIVE (B): Other species (Non falciparum): In addition to the control band, one pink / pink-purple band appears only at region 'Pan' in the test window 'T'.



6. The test result should not be interpreted after 15 minutes.

7. The test should be considered invalid if no bands appear on the device. Repeat the test with a new device ensuring that the test procedure has been followed accurately.

LIMITATIONS OF THE TEST

1. As with all diagnostic tests, the test result must always be correlated with clinical findings.
2. The results of test are to be interpreted within the epidemiological, clinical and therapeutic context. When it seems indicated, the parasitological techniques of reference should be considered (microscopic examination of the thick smear and thin blood films).
3. Any modification to the above procedure and / or use of other reagents will invalidate the test procedure.
4. The device and buffer of different lots must not be mixed and used.
5. In case of mixed infection (*P. falciparum* with other malarial species), both, Pf and pan malaria band will be positive. Hence differentiation of infection due to *P.vivax*, *P.ovale* or *P.malariae* cannot be done.
6. While monitoring therapy, using the pan band, if the reaction of the test remains positive with the same intensity after 5-10 days, post treatment, the possibility of a resistant strain of malaria has to be considered.
7. Usually, the pan band turn negative after successful anti malarial therapy. However, since treatment duration and medication used affect the clearance of parasites, the test should be repeated after 5-10 days of start of treatment.
8. In *P. falciparum* malaria infection, HRP-2 is not secreted in the gametogony stage. Hence, in "Carriers", the HRP-2 band may be absent.

9. HRP-2 levels, post treatment persist up to 15 days, the pan band can be used to monitor success of therapy, in *P. falciparum* malaria cases.
10. In a few cases, where the HRP-2 band is positive and the pan malaria band is negative, it may indicate a case of post treatment malaria. However, such a reaction pattern may also be obtained in a few cases of untreated malaria. Retesting after 2 days is advised, in such cases.

PERFORMANCE CHARACTERISTICS

In an in-house study, a panel of 251 samples whose results were earlier confirmed with microscopy was tested with **FirstSign™ – ParaView**. The results obtained are as follows:

Sample	Total No. of samples tested	FirstSign™ – ParaView		Sensitivity	Specificity
		Positive	Negative		
<i>P. falciparum</i> +Ve	16	16	0	100%	-
<i>P. vivax</i> +Ve	25	25	0	100%	-
Malaria -Ve	210	210	210	-	100%

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13. Data on file: Unimed International, Inc., USA.



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ParaView – 2

Rapid Test for Malaria – Pv + Pf

A rapid Immunochromatographic test for the qualitative detection and differentiation of Plasmodium vivax (Pv) and Plasmodium falciparum (Pf) in Whole blood

INTRODUCTION

Unimed **FirstSign™ – ParaView - 2** is a self-performing, qualitative, sandwich immunoassay for the detection and differentiation of vivax malaria and falciparum malaria in whole blood samples.

SUMMARY

Four species of the Plasmodium parasites are responsible for malaria infections in human viz. *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. Of these *P. falciparum* and *P. vivax* are the most prevalent. Early detection and differentiation of malaria is of paramount importance due to incidence of cerebral malaria and drug resistance associated with falciparum malaria causing most of the morbidity and mortality worldwide. **FirstSign™ – ParaView - 2** is based on the detection of an abundant intracellular metabolic enzyme produced by malarial parasites in the blood. The enzyme, Lactate DeHydrogenase (pLDH), is released from viable parasitized blood cells and is rapidly detected by a series of monoclonal antibodies. Differentiation between malarial species is based on antigenic differences between pLDH isoforms. Since the pLDH is the product of viable parasites the test may be used to monitor effective antimalarial therapy.

FirstSign™ – ParaView - 2 detects the presence of vivax specific pLDH and falciparum specific pLDH in whole blood specimen and is a sensitive and specific test for the detection and differentiation of vivax malaria and falciparum malaria.

PRINCIPLE

FirstSign™ – ParaView - 2 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 anti pan specific pLDH colloidal gold conjugate (monoclonal) antisera complexes the 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) antisera and/ or the anti falciparum specific pLDH coated on the membrane leading to formation of 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 purple band. This control band serves to validate the test performance.

REAGENTS AND MATERIAL SUPPLIED

FirstSign™ – ParaView - 2 kit contains:

1. Individually pouched devices: Membrane assembly predisposed with anti pan specific pLDH-colloidal gold conjugated antisera, rabbit antisera conjugated colloidal gold and anti vivax specific pLDH antisera, anti falciparum specific pLDH antisera, anti rabbit antisera at the respective regions and a sample loop.
2. Clearing buffer in a dropper bottle.

OPTIONAL MATERIAL REQUIRED

Calibrated micropipette capable of delivering 5ul sample accurately.

STORAGE AND STABILITY

The test kit may be stored between 4 - 30°C till the duration of the shelf life as indicated on the pouch / carton. DO NOT FREEZE.

NOTE

Read the instructions carefully before performing the test.

For in vitro diagnostic use only. NOT FOR MEDICINAL USE.

Do not use beyond expiry date.

Do not inter mix reagents from different lots.

Handle all specimens as potentially infectious.

Follow standard biosafety guidelines for handling and disposal of potentially infective material and kit materials.

SPECIMEN COLLECTION AND PREPARATION

Fresh blood from finger prick / 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 collected in a clean glass or plastic container. If immediate testing is not possible then the specimen may be stored at 2– 8°C for up to 72 hours before testing. Clotted or contaminated blood samples should not be used for performing the test. Fresh blood from finger prick / puncture may also be used as a test specimen.

INSTRUCTIONS FOR USE

1. Bring the kit components to room temperature before testing.
2. In case the pouch has been stored at 2– 8°C, allow at least 30 minutes for the device to come to room temperature. Check the color of the desiccant. It should be blue. If it has turned colorless or faint blue, discard the device and use another device.
3. Open the pouch and remove the device. Once opened, the device must be used immediately.
4. Evenly mix the anti coagulated blood sample by gentle swirling. Dip the sample loop in to the sample.
Blot the blood so collected on to the sample pad in the sample well 'S'. (This delivers approximately 5 µl of the whole blood specimen).

OR

In case finger prick blood is being used, touch the sample loop to the blood on the finger prick and immediately blot the specimen on to the sample pad in the sample well 'S' (Care should be taken that the blood sample has not clotted and the transfer to the sample pad is immediate).

OR

Alternatively, 5µl of the anti coagulated or finger prick specimen may be delivered to the sample pad in the sample well 'S' using a micropipette.

NOTE: Ensure the blood from the sample loop has been completely taken up by the sample pad.

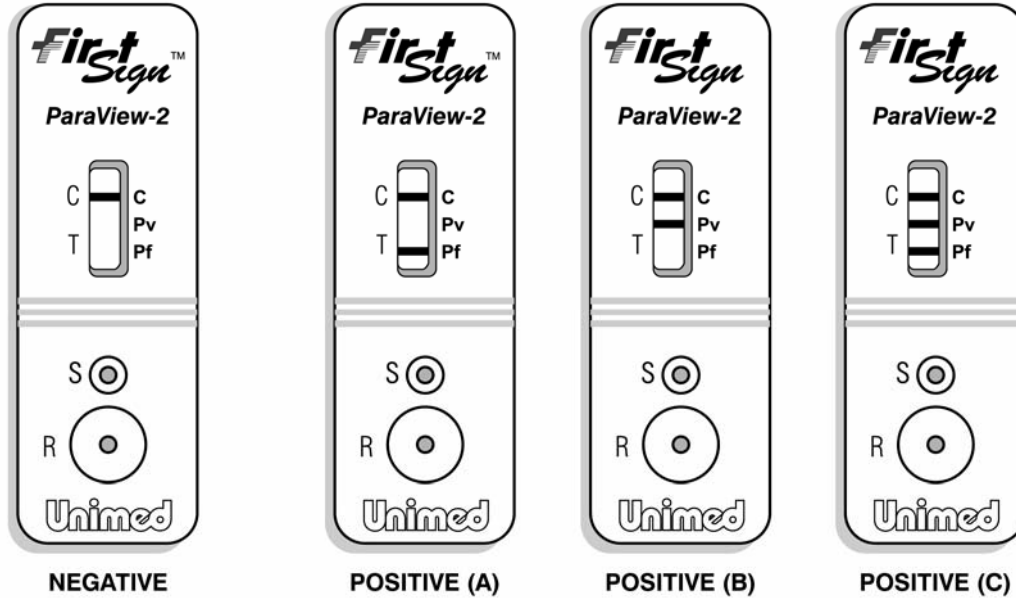
5. Dispense six drops of the clearing buffer into well 'R', by holding the plastic dropper bottle vertically.
6. At the end of 15 minutes, read the results as follows:

NEGATIVE for malaria :Only one colored band appears in the control window ‘C’.

POSITIVE (A) for *P.falciparum* malaria: In addition to the control band, a distinct colored band also appears under the region marked Pf in the Test window ‘T’.

POSITIVE (B) for *P.vivax* malaria: In addition to the control band, a distinct colored band also appears under the region marked Pv in the Test window ‘T’

POSITIVE (C) for *P.falciparum* and *P.vivax* malaria: In addition to the control band, distinct colored bands appear under the region marked Pf and Pv in the Test window ‘T’.



8. The test should be considered invalid if no bands appear on the device. Repeat the test with a new device ensuring that the test procedure has been followed accurately.

LIMITATIONS OF THE TEST

1. As with all diagnostic tests, the results must always be correlated with clinical findings.
2. **FirstSign™ – ParaView - 2** is 100% sensitive to *P. falciparum* and *P. vivax* malaria.

PERFORMANCE CHARACTERISTICS

In an in-house study a panel of 207 samples whose results were earlier confirmed with microscopy were tested with **FirstSign™ – ParaView - 2**. The results obtained are as follows:

Sample	Total No. of samples tested	<i>FirstSign™ – ParaView - 2</i>		Sensitivity	Specificity
		Positive	Negative		
P. Falciparum +Ve	22	22	0	100%	-
P. Vivax +Ve	17	17	0	100%	-
Malaria -Ve	168	0	168	-	100%

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1. Makler, M. T., et. al. (1993) Parasite lactate assay as an assay for *Plasmodium falciparum* drug sensitivity. Am. J. Trop. Med. Hyg. 48(6), 739-741.
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4. Hunte-Cooke A., et. al., (1999) Comparison of a Parasite Lactate Dehydrogenase-based Immunochromatographic Antigen Detection assay (OptiMAL®) with Microscopy for the Detection of Malaria Parasites in Human Blood Samples. Am J. Trop Med 60(2). 173-176.
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8. Moody A., et. al (2000) Performance of the OptiMAL® malaria antigen capture dipstick for malaria diagnosis and treatment monitoring. British Journal of Hematology, 109, 1-5





ParaView – 3

Rapid Test for Malaria – Pan / Pv / Pf

A rapid Immunochromatographic test for the qualitative detection P.falciparum specific histidine rich protein-2 (Pf HRP-2), P.vivax specific pLDH and Pan malaria specific pLDH in Whole blood

INTRODUCTION

Unimed **FirstSign™ – ParaView - 3** is a rapid self-performing, qualitative, two site sandwich immunoassay utilizing whole blood for the detection of *P.falciparum* specific histidine rich protein-2 (Pf HRP-2), *P.vivax* specific pLDH and pan malaria specific pLDH. The test can be used for the specific detection of *P. falciparum* and *P.vivax* malaria, differentiation of other malarial species and for the follow up of antimalarial therapy.

SUMMARY

Four species of the Plasmodium parasites are responsible for malaria infections in human viz. *P. falciparum*, *P.vivax*, *P.ovale* and *P.malariae*. Of these, *P. falciparum* and *P.vivax* are the most prevalent. Early detection and differentiation of malaria is of utmost importance due to incidence of cerebral malarial and drug resistance associated with falciparum malaria and due to the morbidity associated with the other malarial forms. As the course of treatment is dependent on the species, differentiation between *P. falciparum* and *P.vivax* is of utmost importance for better patient management and speedy recovery.

In **FirstSign™ – ParaView - 3** the detection system for *P. falciparum* malaria is based on the detection of *P. falciparum* specific histidine rich protein –2 (Pf HRP-2), which is a water-soluble protein that is released from parasitised erythrocytes of, infected individuals. The detection system of *P.vivax* is based on the presence of *P.vivax* specific pLDH. Further the detection of other malarial infections such as *P.ovale* and *P.malariae* is achieved through the pan malaria specific pLDH.

Since pLDH is a product of viable parasites, the pan band may also be used to monitor course of effective antimalarial therapy.

FirstSign™ – ParaView - 3 detects the presence of *P. falciparum* specific Pf.HRP-2, *P.vivax* specific pLDH and pan specific pLDH in whole blood specimen and is a sensitive and specific test for the detection of all malaria species, differentiation for *P. falciparum* and *P.vivax* and monitoring successful antimalarial therapy.

PRINCIPLE

FirstSign™ – ParaView - 3 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 anti-HRP-2 antibody, anti *P.vivax* specific pLDH antibody and anti pan specific pLDH antibody complexes the HRP-2 / corresponding pLDH in the lysed sample. This complex moves further on the membrane to the test region where it is immobilized by the monoclonal anti Pf. HRP-2 antibody and / or monoclonal anti *P.vivax* specific pLDH antibody and / or monoclonal pan specific pLDH antibody coated on the membrane leading to formation of a pink / purple colored band in the respective regions which confirms a positive test result. Absence of a colored band in the test region indicates a negative test result for the corresponding antigen. The unreacted conjugate along with the rabbit antisera colloidal gold 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. This control band serves to validate the test performance.

REAGENTS AND MATERIAL SUPPLIED

FirstSign™ – ParaView - 3 kits contains:

A. Individual pouches, each containing:

1. Test Device: Membrane assembly pre-dispensed with monoclonal anti- HRP-2 antibody–colloidal gold conjugate, monoclonal anti *P. vivax* specific pLDH antibody-colloidal gold conjugate, monoclonal anti pan specific pLDH antibody-colloidal gold conjugate, rabbit globulin colloidal gold conjugate, monoclonal anti Pf. HRP-2 antibody, monoclonal anti *P. vivax* specific pLDH antibody, monoclonal anti pan specific pLDH antibody and anti-rabbit antibody at the respective regions.

2. Desiccant pouch.

3. 5 µl sample loop.

B. Clearing buffer in a dropper bottle.

C. Package insert.

OPTIONAL MATERIAL REQUIRED

Calibrated micropipettes capable of delivering 5 ml sample accurately.

STORAGE AND STABILITY

The test kit may be stored between 4-30°C till the duration of the shelf life as indicated on the pouch / carton. DO NOT FREEZE.

NOTE

Read the instructions carefully before performing the test. For in vitro diagnostic use only. NOT FOR MEDICINAL USE. Do not use beyond expiry date. Do not inter mix reagents from different lots. Handle all specimens as potentially infectious. Follow standard biosafety guidelines for handling and disposal of potentially infective material.

SPECIMEN COLLECTION AND PREPARATION

Fresh anti coagulated whole blood should be used as a test sample and EDTA or Heparin or Oxalate can be used as suitable anticoagulant. The specimen should be collected in a clean glass or plastic container. If immediate testing is not possible then the specimen may be stored at 2–8°C for up to 72 hours before testing. Clotted or contaminated blood samples should not be used for performing the test. Fresh blood from finger prick / puncture may also be used as a test specimen.

TEST PROCEDURE

1. Bring the **FirstSign™ – ParaView - 3** kit components to room temperature before testing.

2. Open the pouch and retrieve the device, sample loop and desiccant. Check the color of the desiccant. It should be blue. If it has turned colorless or pink, discard the device and use another device. *Once opened, the device must be used immediately.*

3. Tighten the vial cap of the clearing buffer provided with the kit in the clockwise direction to pierce the dropper bottle nozzle.

4. Evenly mix the anti coagulated blood sample by gentle swirling. Dip the sample loop into the sample. Ensuring that a loop full of blood is retrieved, blot the blood so collected on to the sample pad in the sample port 'S'. (This delivers approximately 5 µl of the whole blood specimen).

OR

In case finger prick blood is being used, touch the sample loop to the blood on the finger prick. Ensuring that a loop full of blood is retrieved, immediately blot the specimen on to the sample pad in the sample port 'S'. (Care should be taken that the blood sample has not clotted and the transfer to the sample pad is immediate).

OR

Alternatively, 5 µl of the anti coagulated or finger prick specimen may be delivered to the sample pad in the sample port 'S' using a micropipette.

NOTE: Ensure that the blood from the sample loop has been completely taken up by the sample pad.

- Dispense six drops of the clearing buffer into port 'R', by holding the plastic dropper bottle vertically.
- At the end of 15 minutes read the results as follows:

NEGATIVE for malaria:

Only one pink / pink-purple band appears at the control region 'C'.

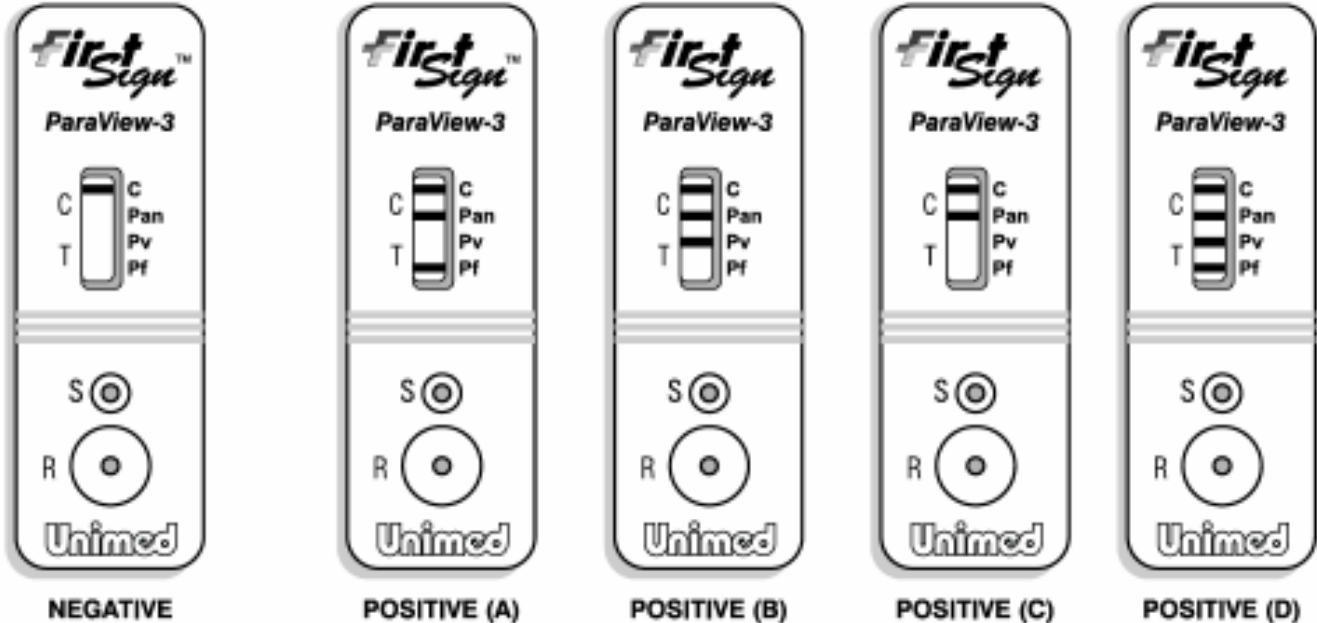
POSITIVE for Malaria:

POSITIVE (A): *P. falciparum* malaria: In addition to the control band, a pink / pink-purple band appears at the 'Pf' and 'Pan' regions respectively.

POSITIVE (B): *P. vivax* malaria: In addition to the control band, a pink / pink-purple band appears at 'Pv' and 'Pan' regions respectively.

POSITIVE (C): Other species: In addition to the control band, one pink / pink-purple band appears only at 'Pan' region.

POSITIVE (D): Mixed infection: In addition to the control band, a pink / pink-purple band appears at 'Pf', 'Pv' and 'Pan' regions respectively.



- The test should be considered invalid if no bands appear on the device. Repeat the test with a new device ensuring that the test procedure has been followed accurately.

LIMITATIONS OF THE TEST

1. As with all diagnostic tests, the test result must always be correlated with clinical findings.
2. The results of test are to be interpreted within the epidemiological, clinical and therapeutic context. When it seems indicated, the parasitological techniques of reference should be considered (microscopic examination of the thick smear and thin blood films).
3. Any modification to the above procedure and / or use of other reagents will invalidate the test procedure.
4. The device and buffer of different lots must not be mixed and used.
5. In case of infection due to *P.vivax* or *P.falciparum*, or due to mixed infection by these species, the pan malaria band will also be positive. Hence differentiation of infection due to *P.ovale* or *P.malariae* cannot be done.
6. While monitoring therapy, if the reaction of the test remains positive with the same intensity after 5-10 days, post treatment, the possibility of a resistant strain of malaria has to be considered.
7. Usually, the Pv and pan bands turn negative after successful anti malarial therapy. However, since treatment duration and medication used affect the clearance of parasites, the test should be repeated after 5-10 days of start of treatment.
8. In *P. falciparum* malaria infection, HRP-2 is not secreted in gametogony stage. Hence, in "Carriers", the HRP-2 band may be absent.
9. HRP-2 levels, post treatment persist up to 15 days, the pan band can be used to monitor success of therapy, in *P. falciparum* malaria cases.
10. In a few cases, where the HRP-2 band is positive and the pan malaria band is negative, it may indicate a case of post treatment malaria. However, such a reaction pattern may also be obtained in a few cases of untreated malaria. Retesting after 2 days is advised, in such cases.

PERFORMANCE CHARACTERISTICS

In an in-house study, a panel of 251 samples whose results were earlier confirmed with microscopy was tested with **FirstSign™ – ParaView - 3**. The results obtained are as follows:

Sample	Total No. of samples tested	FirstSign™ – ParaView - 3		Sensitivity	Specificity
		Positive	Negative		
P. falciparum +Ve	16	16	0	100%	-
P. vivax +Ve	25	25	0	100%	-
Malaria -Ve	210	210	210	-	100%

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Rapid Test for *P. falciparum* Malaria

A rapid Immunochromatographic test for the qualitative detection of Plasmodium falciparum (P. f.) Malaria antigen in whole blood

For Professional In Vitro Diagnostic use only

Read Instructions before use

INTENDED USE

Unimed **FirstSign™ - Malaria P.f.** is a rapid Immunochromatographic assay for the qualitative determination of *P. falciparum* specific histidine-rich protein –2 (Pf HRP-2) in human whole blood.

SUMMARY

There are four species of the Plasmodium parasites, which are responsible for malaria infections in human: *P. falciparum*, *P.vivax*, *P.ovale* and *P.malariae*. *P. falciparum* and *P.vivax* cause 95 % of all human malaria. Of these *P. falciparum* is the most prevalent and severe species that is responsible for most of the morbidity and mortality worldwide. Early detection of *P. falciparum* malaria is of paramount importance due to incidence of cerebral malaria and drug resistance associated with it. Pf HRP-2 is a water-soluble protein that is released from parasitised erythrocytes of infected individuals and is specific to the *P.falciparum* species.

FirstSign™ - Malaria P.f. detects the presence of Pf HRP-2 in whole blood specimen and is a sensitive and specific test for the detection of *P. falciparum* malaria.

PRINCIPLE

FirstSign™ - Malaria P.f. is a rapid test for the detection of *P.falciparum* malaria that utilizes the principle of immunochromatography. As the test sample flows through the membrane assembly of the dipstick after placing into the clearing buffer tube, the colored anti - Pf HRP-2 (monoclonal) - colloidal gold conjugate complexes the Pf HRP-2 in the lysed sample. This complex moves further on the membrane to the test region where it is immobilized by the anti - Pf HRP-2 (monoclonal) coated on the membrane leading to formation of a pink colored line which confirms a positive test result. Absence of this colored line 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 - mouse antibodies coated on the membrane at the control region, forming a pink line. This control line serves to validate the test performance.

REAGENTS AND MATERIALS PROVIDED

1. Card Test: Individually pouched Card test with a desiccant and a 5 µl sample loop.
2. Reagent 1 (in a plastic dropper bottle).
3. Product Insert

MATERIALS REQUIRED NOT INCLUDED (OPTIONAL)

Calibrated micropipette capable of delivering 5µl sample accurately .

STORAGE AND STABILITY

The test kit may be stored between 4° to 30°C till the duration of the shelf life as indicated on the Pouch / Box. DO NOT FREEZE.

Notes

- Read the instructions carefully before performing the test.
- For professional in vitro diagnostic use only. NOT FOR MEDICINAL USE.
- Do not use the test beyond expiry date.
- Do not inter mix reagents from different kit lots.
- Handle all specimens as potentially infectious.
- Follow standard biosafety guidelines for handling and disposal of potentially infective material.

SPECIMEN COLLECTION AND PREPARATION

1. Fresh anti coagulated whole blood should be used as a test sample. EDTA or Heparin or Oxalate may be used as a suitable anticoagulant. The specimen should be collected in a clean glass or plastic container. Fresh blood from finger

Product Insert (Card Test)

prick / puncture may also be used as a test specimen.

2. If immediate testing is not possible then the specimen may be stored at 2° to 8°C for up to 72 hours before testing. Clotted or contaminated blood samples should not be used for performing the test.

TEST PROCEDURE

1. Bring the **FirstSign™ - Malaria P.f.** kit components to room temperature before testing.
2. In case the pouch has been stored at 2 – 8°C, allow at least 30 minutes for the device to come to room temperature.
3. Open the pouch and remove the device, sample loop and desiccant. Check the color of the desiccant. It should be blue. If it has turned colorless or faint blue, discard the device and use another device. *Once opened, the device must be used immediately.*
4. Tighten the vial cap of the clearing buffer provided with the kit in the clockwise direction to pierce the dropper bottle nozzle.
5. Evenly mix the anti coagulated blood sample by gentle swirling. Dip the sample loop into the sample. Ensuring that a loop full of blood is retrieved, blot the blood so collected on to the sample pad in the sample port 'S'. (This delivers approximately 5 µl of the whole blood specimen)

OR

In case finger prick blood is being used, touch the sample loop to the blood on the finger prick. Ensuring that a loop full of blood is retrieved, immediately blot the specimen on to the sample pad in the sample port 'S' (Care should be taken that the blood sample has not clotted and the transfer to the sample pad is immediate).

OR

Alternatively, 5 µl of the anticoagulated or finger prick specimen may be delivered to the sample pad in the sample port 'S' using a micropipette.

NOTE : Ensure the blood from the sample loop has been completely taken up by the sample pad.

6. Dispense six drops (300 µl) of the Reagent 1 into well 'R', by holding the plastic dropper bottle vertically.
7. Read results at the end of 15 minutes.
8. The test results should not be interpreted after 15 minutes.

INTERPRETATION OF RESULTS

NEGATIVE:

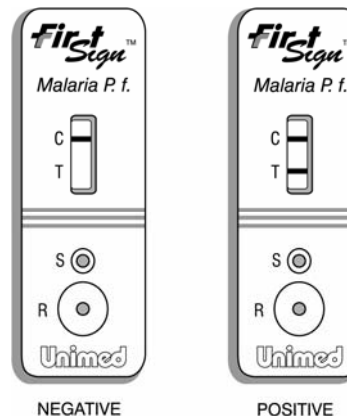
Only one pink colored band appears in the control window 'C'.

POSITIVE:

In addition to the control band, a distinct pink colored band also appears in the Test window 'T'.

INVALID:

The test should be considered invalid if no bands appear on the device. Repeat the test with a new device ensuring that the test procedure has been followed accurately.



LIMITATIONS OF THE TEST

1. Since the Pf HRP-2 persists for up to a fortnight even after successful therapy, a positive test result does not indicate a failed therapeutic response.
2. In case the test needs to be used to monitor success of therapy, testing is advised only from 15 days after the completion of therapy.
3. As with all diagnostic tests, the results must always be correlated with clinical findings.

PERFORMANCE CHARACTERISTICS

1. In an independent study, 125 patient samples from a *P. falciparum* endemic area were tested with **FirstSign™ - Malaria P.f.** and microscopy (thick and thin smear). **FirstSign™ - Malaria P.f.** was found to be 100% sensitive and 100% specific to *P. falciparum* against microscopy. All the 31 samples that tested positive for *P. falciparum* under microscopy showed positive results with **FirstSign™ - Malaria P.f.** The four *P. vivax* positive samples and the 90 malaria negative samples tested negative with **FirstSign™ - Malaria P.f.**
2. In a separate independent study with a panel of 167 samples (74 samples positive for *P. falciparum*, 8 samples positive for *P. vivax*, and 85 Malaria negative samples), **FirstSign™ - Malaria P.f.** demonstrated a sensitivity of 98.8% and specificity of 100% when compared with microscopic diagnosis of thick blood films. There

Product Insert
(Card Test)

was no cross reactivity with *P. vivax* blood specimens.

3. From the above studies and the results of in house data, **FirstSign™ - Malaria P.f.** is a highly sensitive and specific test for the diagnosis of *P. falciparum* malaria.

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