CE

FalciVax™

REF	503010010	503010025	503010050	503010100

RAPID TEST FOR MALARIA
Pv/Pf
DEVICE

INTENDED USE
FalciVax™ is a rapid, qualitative, two site sandwich immunoassay utilizing capillary and venous whole blood specimens of symptomatic patients for the detection of *P.falciparum* specific histidine rich protein-2 (Pf. HRP-2) and *P.vivax* specific plasmodium Lactate Dehydrogenase (pLDH) antigens and it is used in aiding the diagnosis and differentiation of malaria infections caused by *P.falciparum* and *P.vivax*.
It is intended to be used by trained healthcare or laboratory professionals or other health care workers who have received appropriate training. This product can be used by trained lay providers operating at point-of-care in resource-limited settings. This product is not intended for self-testing and it is not for blood donor screening. The test is not automated; it needs to be performed and interpreted manually by the user.

SUMMARY
Four species of the Plasmodium parasites are responsible for malarial infections in human viz. *P. falciparum* , *P.vivax*, *P.ovale* and *P.malariae*. Of these *P. falciparum* and *P. vivax* are considered the "Big Two" due to incidence of cerebral malaria and drug resistance associated with *P. falciparum* malaria, and high rate of infectivity and relapse associated with *P.vivax*. 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 ***FalciVax***™**,** 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 red blood cells of infected individuals. The detection system for *P.vivax* malaria is based on presence of *P.vivax* specific pLDH.

PRINCIPLE
FalciVax™ utilizes the principle of agglutination of antibodies/ antisera with respective antigen in immuno-chromatography format along with use of nano gold particles as agglutination revealing agent. As the test specimen flows through the membrane assembly of the device after addition of the clearing buffer, the colored colloidal gold conjugates of the Agglutinating Sera for HRP-2 and the Agglutinating Sera for *P. vivax* specific pLDH complexes the HRP-2/ pLDH in the lysed specimen. This complex moves further on the membrane to the test region where it is immobilized by the Agglutinating Sera for Malaria specific pLDH and / or Agglutinating Sera for HRP-2 coated on the membrane leading to formation of pink-purple colored band/s which confirms a positive test result. A band will appear under Pf at the test region in falciparum positive specimens, while a band will appear under Pv in vivax malaria positive specimens. 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 Agglutinating Sera for Rabbit globulin coated on the membrane at the control region, forming a pink-purple band. The control band formation is based on the 'Rabbit globulin / Agglutinating Sera for Rabbit globulin' system. Since it is completely independent of the analyte detection system, it facilitates formation of consistent control band signal independent of the analyte concentration. This control band serves to validate the test performance.

REAGENTS AND MATERIALS SUPPLIED
FalciVax™ kit contains:
A. Individual pouches, each containing:
   1. DEVICE Membrane assembly pre-dispensed with Agglutinating Sera for HRP-2 - colloidal gold conjugate, Agglutinating Sera for *P. vivax* specific pLDH - colloidal gold conjugate, rabbit globulin colloidal gold conjugate, Agglutinating Sera for HRP-2, Agglutinating Sera for Malaria specific pLDH and Agglutinating Sera for Rabbit globulin at the respective regions.
   2. Desiccant pouch.
   3. PIPETTE Disposable Plastic Specimen Applicator.
B. BUF Clearing buffer in a dropper bottle.
C. Instructions for use.
D. Pictorial instructions for use.
E. Alcohol swabs – 70% Isopropyl alcohol.
F. Sterile lancets.

Product codes REF	503010010	503010025	503010050	503010100
Pouch sealed tests Σ	10	25	50	100
Clearing buffer bottles	01 x 3.0ml	01 x 4.0ml	02 x 4.0ml	04 x 4.0ml
Alcohol swabs	10	25	50	100
Sterile lancets	10	25	50	100
Instructions for use	01	01	01	01
Pictorial instructions for use	01	01	01	01

MATERIALS REQUIRED BUT NOT PROVIDED
Calibrated micropipette capable of delivering 5µl specimen accurately, disposable micropipette tips.
Permanent marker Pen/pencil, disposable gloves, timer.
Biosafety sharps container and Biohazard waste container (for potentially infectious waste).
Venipuncture blood collection kit (if whole blood is collected by venepuncture).
Additional alcohol swabs (if any included in the kit are found dry) and additional sterile lancets (if any included in the kit have the sterility seal broken).

STORAGE AND STABILITY
The sealed pouches in the test kit & the kit components may be stored between 1°C to 40°C till the duration of the shelf life as indicated on the pouch/ carton. DO NOT FREEZE. After first opening of the clearing buffer bottle, it can be stored between 1°C to 40°C for the remaining duration of its shelf life.

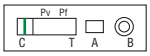
WARNINGS
Read the instructions carefully before performing the test.
For in vitro diagnostic use only. NOT FOR MEDICINAL USE. For professional use.
The test is for aiding in diagnosis of malaria infection and not for screening which requires confirmation.
Do not use beyond expiry date.
Do not use components from different lots of the product.
The device, specimen applicator, alcohol swab and blood lancet are for single use only.
Contact with the contents of desiccant pouch containing, among other substances, cobalt chloride (CAS# 7646-79-9) should be kept to a minimum. Inhalation / swallowing may cause harm.
Handle all specimens as potentially infectious.
Follow standard biosafety guidelines for handling and disposal of potentially infectious material.
Clearing buffer contains Sodium Azide(0.1%), avoid skin contact with this reagent. Azide may react with lead and copper in the plumbing system and form highly explosive metal oxides. Flush with large volumes of water to prevent azide build up in the plumbing.

SPECIMEN COLLECTION AND PREPARATION
For specimen collection, refer to pictorial instructions for use.
Fresh capillary/venous whole blood from finger prick / puncture should be used as a test specimen. However, fresh anti-coagulated venous whole blood may also be used as a test specimen. Using standard blood collection practices, collect venous whole blood into the commercially available anti-coagulant tube such as EDTA or CPDA or Heparin or Oxalate or Tri-sodium Citrate. If immediate testing is not possible then the specimen may be stored at 2°C to 8°C for upto 72 hours before testing and should be brought to room temperature (20°C to 30°C) before use on the test. Clotted, hemolysed or lipaemic whole blood specimens should not be used for performing the test.

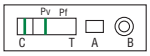
TESTING PROCEDURE AND INTERPRETATION OF RESULTS

1. Bring the ***FalciVax***™ kit components to room temperature (20°C to 30°C) before testing.
2. Open the pouch and retrieve the device, specimen applicator and desiccant pouch. 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. Label the test device with patient identifier.
4. Place the testing device on a flat horizontal surface.
5. Tighten the cap of the clearing buffer bottle provided with the kit in the clockwise direction to pierce the buffer bottle nozzle.
6. **Specimen application**
   - **6.1 Venous whole blood:** Evenly mix the anti-coagulated whole blood by gentle swirling. Dip the specimen applicator into the whole blood. Ensuring that an applicator full of blood is retrieved, immediately blot the blood so collected in the specimen port 'A'(This delivers approximately 5µl of the whole blood specimen). Alternatively, 5µl of the anti-coagulated venous whole blood specimen may be delivered in the specimen port 'A' using a micropipette.
   - **6.2 Capillary whole blood:** Touch the specimen applicator to the whole blood on the finger prick. Ensuring that an applicator full of blood is retrieved, immediately blot the blood so collected in the specimen port 'A'(Care should be taken that whole blood specimen is not clotted and transfer to the specimen port is immediate). Alternatively, 5µl of the capillary finger-prick whole blood specimen may be delivered in the specimen port 'A' using a micropipette.

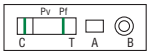
   Note: Ensure that the whole blood from the specimen applicator has been completely taken up at the specimen port 'A'.
7. Immediately dispense **two drops** of clearing buffer into buffer port 'B' holding the buffer bottle vertically and switch on the timer. To avoid contamination of clearing buffer bottle, do not touch the buffer port 'B' with the tip of clearing buffer bottle.
8. Read the results at the end of **20 minutes** as follows:



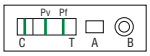
NEGATIVE for malaria : Only one pink-purple band appears in the control window 'C'.



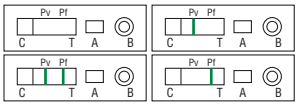
POSITIVE for *P.vivax* malaria : In addition to the control band, a pink-purple band also appears under the region marked 'Pv' in the test window 'T'. Appearance of a coloured band of any intensity (faint to dark) at 'Pv' should be considered as positive result for *P.vivax* malaria.



POSITIVE for *P.falciparum* malaria : In addition to the control band, a pink-purple band also appears under the region marked 'Pf' in the test window 'T'. Appearance of a coloured band of any intensity(faint to dark) at 'Pf' should be considered as positive result for *P.falciparum* malaria.



POSITIVE for *P.falciparum* and *P.vivax* malaria : In addition to the control band, two pink-purple bands appear under the regions marked 'Pf' and 'Pv' in the test window 'T'. Appearance of coloured bands of any intensity (faint to dark) at 'Pf' and 'Pv' should be considered as positive results for *P.falciparum* and *P.vivax* malaria.



INVALID RESULT: The test should be considered invalid if no bands appear on the device. The test should also be considered invalid if only test bands (Pv and/or Pf) appear and no control band appears. Repeat the test with a new device ensuring that the test procedure has been followed accurately.

CAUTION : Do not read results after 30 minutes as it may show erroneous results.

PERFORMANCE CHARACTERISTICS

A. Analytical Performance Study

A1. Potentially interfering exogenous and endogenous substances :

The following Potentially interfering substances have no impact on test results of ***FalciVax***™ :

Type of Specimen		Sr. No.	Potential Interfering substances
Endogenous substance		1	Total Protein
		2	Bilirubin, conjugated
		3	Cholesterol
		4	Triglycerides
		5	Haemoglobin
Common Drugs	Antibiotic	1	Amoxicillin
		2	Ciprofloxacin
	Anti-inflammatory	1	Aspirin
		2	Ibuprofen
Exogenous Substance	Anti-Malaria Drugs	1	Chloroquine
		2	Doxycycline
		3	ACT
		4	Primaquine
		5	Mefloquine
		6	Sulfadoxine
		7	Pyrimethamine
	Anti-TB Drugs	1	Ethambutol
		2	Isoniazide
		3	Rifampin
	Anti-Retroviral Drugs	1	Lamivudine
		2	Efavirenz
		3	Emtricitabine
		4	Tenofovir
		5	Atazanavir

A2. Cross Reacting infections, disease and medical conditions:

The following 17 potential cross reacting infections/diseases/conditions did not affect the performance of ***FalciVax***™.

Potential Cross reacting infections/diseases/conditions			
1	*T. cruzi*	10	*Toxoplasma gondii*
2	Dengue virus	11	Influenza A/B
3	*Leishmania spp*	12	Yellow fever virus
4	*Brucella spp*	13	*Leptospira spp*
5	Measles virus (Rubeola virus)	14	*Treponema pallidum*
6	HAV	15	HAMA
7	HBV	16	ANA
8	HCV	17	Rheumatoid factor
9	HIV-1/HIV-2		

A3. Precision (Repeatability)

Within run, precision was determined using 10 replicates of 5 different venous whole blood specimens in 03 different lots of ***FalciVax***™ which is summarized below:

*Quality control Panel	Accuracy (%)
Malaria Negative	100%
P.falciparum Positive (Moderate Positive)	100%
P.falciparum Positive (Weak Positive)	100%
P.vivax Positive (Moderate Positive)	100%
P.vivax Positive (Weak Positive)	100%

A4. Precision (Reproducibility)

Between run, precision was determined using 5 different blinded venous whole blood specimens in 3 different lots of ***FalciVax***™ X 3 different operators X 3 different sites X 5 different days which is summarized below:

*Quality control Panel	Accuracy (%)			
	Between Day	Between Operator	Between lot	Between site
Malaria Negative	100%	100%	100%	100%
P.falciparum Positive (Moderate Positive)	100%	100%	100%	100%
P.falciparum Positive (Weak Positive)	100%	97.7%	100%	100%
P.vivax Positive (Moderate Positive)	100%	100%	100%	100%
P.vivax Positive (Weak Positive)	100%	100%	100%	100%

*Quality control panel specimens have been confirmed by microscopy as malaria negative and malaria positive. Malaria positive specimens were classified as moderate or weak positive based on respective parasite counts as determined by microscopy.

A5. Analytical Sensitivity

The sensitivity of ***FalciVax***™ for *P.falciparum* is 100 parasites/µl and for *P.vivax* is 200 parasite/µl based on microscopy results.

B. Clinical Performance study: Diagnostic Specificity and Diagnostic Sensitivity

B1. In an in-house study, a panel of 200 venous whole blood specimens whose results were earlier confirmed with microscopy were tested with ***FalciVax***™. The results obtained are as follows:

Specimens	Total no. of specimens tested	***FalciVax***™		Sensitivity (95% CI)	Specificity (95% CI)
		Positive	Negative		
P.falciparum	20	20	0	100% (83.16% to 100.00%)	-
P.vivax	25	25	0	100% (86.28% to 100.00%)	-
Pf and *Pv* Malaria Negative	155	0	155	-	100% (97.65% to 100.00%)

B2. External evaluation studies:

Table 1

Study Site	Total Number of Malaria Negative specimens Tested	Specimen Type		Number of specimens Negative by Microscopy	Number of specimens Negative in ***FalciVax***™	Number of specimens falsely Positive in ***FalciVax***™
		Population type	Mode of Collection			
Jharkhand, India	985	Hospitalized Patients	Finger prick/ venous phlebotomy	985	985	0
Maharashtra, India.	1000	Blood Donors	Venous whole blood	1000	1000	0
Goa, India.	39	Symptomatic/ Asymptomatic Individuals	Capillary Whole Blood	39	39	0
			Venous Whole Blood	39	39	0
Odisha, India	545	Pregnant Women	Venous Whole Blood	545	545	0
Odisha, India	497	Neonates	Heel Prick	497	497	0

Based on above data:

Total Nos. tested	Overall Specificity	95% Confidence Interval
3105	100%	99.88% to 100.00%

Table 2

Study Site	Total Number of Malaria Positive specimens Tested	Population type	Specimen Type		Number of specimens Positive by Microscopy	Number of specimens Positive in ***FalciVax***™	Number of specimens falsely Negative in ***FalciVax***™
			Mode of Collection	Species Type			
India	403	Hospitalized Patients	Finger prick/ venous phlebotomy	*P.falciparum*	403	403	0
	312			*P.vivax*	312	312	0
	26	Symptomatic/ Asymptomatic Individuals	Capillary and Venous Whole Blood	*P.falciparum*	26	26	0
	29			*P.vivax*	29	29	0
	06			*P.falciparum* + *P.vivax*	06	06	0
	01	Pregnant Women	Venous Whole Blood	*P.falciparum*	01	01	0
	04			*P.vivax*	04	04	0
	01	Neonates	Heel Prick	*P.falciparum*	01	01	0
	02			*P.vivax*	02	02	0

Based on above data:

Plasmodium species	Total Nos. tested	Overall Sensitivity	95% Confidence Interval
P.falciparum	431	100%	99.15% to 100.00%
P.vivax	347	100%	98.94% to 100.00%
P.falciparum + *P.vivax*	06	100%	54.07% to 100.00%

LIMITATIONS OF THE TEST

1. As with all diagnostic tests, the results must always be correlated with clinical findings.
2. The results of test are to be interpreted within the epidemiological, clinical and therapeutic context.
3. Any modification to the above procedure and / or use of other reagents will invalidate the test procedure.
4. Hook effect may be observed at parasite density ≥3,00,000 parasite/µl. In such cases, repeat the test by using different dilutions of same specimen. Other clinical data (e.g symptoms, travel history, risky factors) should be used in conjunction with the test results.
5. Potential cross-reacting diseases such as HAT, Tick-borne Encephalitis and those caused by *Schistosoma spp* have not been tested in this product, and their associated interference in ***FalciVax***™ is not known.
6. Interference due to presence of heterophile antibodies in patient's specimen can lead to erroneous analyte detection in immunoassay, has been reported in various studies. ***FalciVax***™ uses HETEROPHILIC BLOCKING REAGENT (HBR) to inhibit majority of these interferences.
7. In case of (Pv+Pf) mixed infections, ***FalciVax***™ detects *P.vivax* as low as 200 parasite/µl even in presence of high *P.falciparum* densities of ~2,00,000 parasite/µl. In suspected cases of *P. falciparum* densities > 2,00,000 parasite/µl, confirm the results with microscopy.
8. ***FalciVax***™ is 100% sensitive to *P. falciparum* and *P. vivax* malaria. However, a negative test result does not rule out the possibility of infection with *P.ovale* and *P. malariae*.
9. In *P. falciparum* malaria infection, Pf. HRP-2 is not secreted in gametogony stage. Hence in "Carriers", the 'Pf' band may be absent.
10. Since Pf. HRP-2 persists for upto a fortnight even after successful therapy, a positive test result does not indicate a failed therapeutic response. 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.

WARRANTY

This product is designed to perform as described on the label and Instructions for use. The manufacturer disclaims any implied warranty of use and sale for any other purpose. In the event of performance changes or product malfunction, please contact manufacturer.

BIBLIOGRAPHY

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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.
9. Data on file: Zephyr Biomedicals.

SYMBOL KEYS

Temperature Limitation	Manufacturer	DEVICE Device	EC REP Authorised Representative in the European Community
Use by	Consult Instructions for use	PIPETTE Disposable Plastic Specimen Applicator	
Date of Manufacture	REF Catalogue Number	BUF Clearing Buffer	Xn NaN., R22, S23-46-61 Harmful if swallowed. Do not breathe vapour. If swallowed, seek medical advice immediately and show this container or label. Avoid release to the environment. Refer to special instructions.
LOT Batch Number / Lot Number	IVD *In vitro* Diagnostic Medical Device	This side up	
Σ Contains sufficient for <n> tests	Do not reuse	Do not use if package is damaged	

Manufactured by:

Zephyr Biomedicals

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EC REP

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