Size : 137 x 218 mm

PyroCHECK™

Rapid test for the simultaneous detection of Malaria Pv/Pf antigen, IgM antibodies to *S.typhi* and Dengue NS 1 antigen in whole blood

DEVICE

INTENDED USE

PyroCHECK™ is a rapid, qualitative immunochromatographic test for simultaneous detection of *P. falciparum* specific histidine rich protein-2 (Pf. HRP-2) and *P. vivax* specific pLDH, IgM antibodies to *S. typhi* and Dengue NS 1 antigen in human whole blood.

SUMMARY

Five species of the Plasmodium parasites are responsible for malarial infections in human viz. *P falciparum, P. vivax, P. ovale, P. malariae* and *P. Knowlesi*. 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 **PyroCHECK**™ the detection system for *P. falciparum* malaria is based on the detection of *P. falciparum* specific histidine rich protein-2 (Pf.HRP-2) and the detection system for *P. vivax* malaria is based on presence of *P. vivax* specific pLDH.

Typhoid fever is a bacterial infection caused by Salmonella serotypes including *S. typhi, S. paratyphi A, S. paratyphi B* and *Salmonella sendai*. The symptoms of the illness include high fever, headache, abdominal pain, constipation and appearance of skin rashes. Accurate diagnosis of typhoid fever at an early stage is not only important for etiological diagnosis but to identify and treat the potential carriers and prevent acute typhoid fever outbreaks. Early rising antibodies to Lypopolysaccharide (LPS) O are predominantly IgM in nature. Detection of *S. typhi* specific IgM antibodies would serve as a marker for recent infection. **PyroCHECK**™ qualitatively detects the presence of IgM class of Lypopolysaccharide (LPS) specific to *S. typhi* in human whole blood specimen.

Dengue virus (serotypes 1-4) belongs to the family of Flaviviridae, which is widely distributed in the epidemic and endemic areas throughout tropical and subtropical regions of the world. Dengue virus is transmitted in nature principally by the *Aedes aegypti* and *Aedes albopictus* mosquitoes. Dengue presents typically as a fever of sudden onset with headache, retro-orbital pain, pain in the back and limbs (break-bone fever), lymphadenopathy and maculopapular rash. Primary dengue virus infection is charactrized by elevation in dengue virus specific NS 1 antigen level in patient's blood stream from 1-6 days after onset of symptoms. **PyroCHECK**™ is a new generation rapid Immunochromatographic test for first line detection of Dengue infection in early stage.

PRINCIPLE

PyroCHECK™ 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 sample flows through each membrane assembly of the test device, the colored colloidal gold conjugates of the agglutinating sera for HRP-2 and the agglutinating sera for *P. vivax* specific pLDH, agglutinating sera for human IgM colloidal gold conjugate and the highly specific agglutinating sera for dengue NS 1 colloidal gold conjugate complexes with the HRP-2/pLDH, S. typhi specific IgM antibodies or to Dengue NS 1 antigen respectively present in the specimen. These complexes move further to the test region and get immobilized to give a pink to pink purple coloured band at respective test region. The unreacted conjugate & unbound complex, if any along with the rabbit/mouse globulin-colloidal gold conjugate move further on the membrane and are subsequently immobilized by agglutinating sera for rabbit/mouse globulin coated on the membrane control region forming pink to pink -purple colored band. This control band acts as a procedural control and serves to validate the result.

REAGENTS AND MATERIALS SUPPLIED

PyroCHECK™ kit contains:

A. Individual pouches, each containing:
   1. Test Device comprising of membrane assembly for detection of
      (1) Malaria Pv/Pf
      (2) S.typhi IgM
      (3) Dengue NS 1 Antigen
   2. Desiccant pouch.

B. Sample running buffer in a dropper bottle.

C. Package Insert

D. Disposable plastic specimen applicators
   (1) Inverted cup
   (2) Plastic dropper

REF	507020010	507020025
	10 Tests	25 Tests

OPTIONAL MATERIAL REQUIRED BUT NOT PROVIDED

Calibrated micropipette capable of delivering 5-50 µl specimen accurately, Stop watch.

STORAGE AND STABILITY

The test kit (including sealed pouches) may be stored between 4ºC to 30ºC till the duration of the shelf life as indicated on the pouch / carton. DO NOT FREEZE. After first opening of the sample running buffer bottle, it can be stored between 4ºC to 30ºC for the remaining duration of its shelf life.

NOTES

1. For *in vitro* diagnostic use and for professional use only. NOT FOR MEDICINAL USE.
2. Do not use the kit beyond expiry date and do not re-use the test device.
3. Read the instruction carefully before performing the test.
4. Any modification to the above procedure and / or use of other reagents will invalidate the test procedure.
5. Do not intermix the reagent or devices from different lots.

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6. 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.
7. Handle all specimens as if potentially infectious. Follow standard bio-safety guidelines for handling and disposal of potentially infective material.
8. If desiccant colour at the point of opening the pouch has turned from blue to pink or colorless, another test device must be run.
9. Sample running buffer contains Sodium Azide (0.1%), 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.

SPECIMEN COLLECTION AND PREPARATION

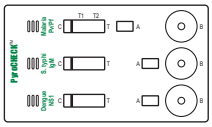
1. No special preparation of the patient is necessary prior to specimen collection by approved techniques.
2. Whole blood collected with a suitable anticoagulant such as EDTA or Heparin or Oxalate should be used as test specimen.
3. The specimen should be collected in a clean glass or plastic container. If immediate testing is not possible then the specimen may be stored at 2°C to 8°C for upto 72 hours before testing. Refrigerated specimens should be brought to Room temperature prior to testing.
4. Repeated freezing, thawing of the specimen should be avoided.
5. Do not use hemolysed, clotted or contaminated specimens.

TESTING PROCEDURE

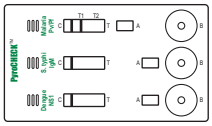
1. Bring the kit components of **PyroCHECK**™ Device to room temperature.
2. Open a foil pouch by tearing along the "notch".
3. Remove the test device and desiccant pouch. Check the color of the desiccant. It should be blue, if it has turned colourless or pink, discard the device and use another device. ***Once opened, the device must be use immediately.***
4. Label the test device with specimen identity.
5. Place the test device on a flat horizontal surface.
6. (a.) For Malaria Pv/Pf and S.typhi IgM : Using the Inverted cup provided in the kit, carefully dispense ~5µl of whole blood at Malaria Pv/Pf and S.typhi IgM specimen port 'A' respectively. Alternatively, 5µl of the whole blood specimen may be delivered in the specimen port 'A' using a micropipette.
   (b.) For Dengue NS 1: Using the plastic dropper provided in the kit, carefully dispense two drops of whole blood at Dengue NS 1 specimen port 'A'. Alternatively, 50µl of the whole blood specimen may be delivered in the specimen port 'A' using a micropipette.
7. Add five drops of sample running buffer into the port "B" of Malaria, Typhoid and Dengue respectively.
8. Read the results at the end of 20 minutes.

INTERPRETATION OF RESULTS

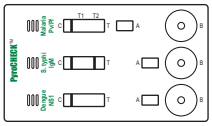
Negative Result: Negative for Malaria Pv/Pf, *S. typhi* IgM & Dengue NS1.



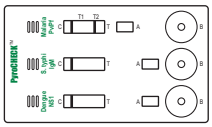
Positive Result



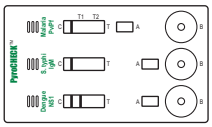
Positive for *P. vivax* malaria & negative for *P. falciparum* malaria, *S.typhi* IgM & Dengue NS1.



Positive for *S. typhi* IgM & Negative for Malaria Pv/Pf & Dengue NS1.



Positive for *P. falciparum* malaria & negative for *P. vivax* malaria, *S.typhi* IgM & Dengue NS1.



Positive for Dengue NS1 & Negative for Malaria Pv/Pf & *S. typhi* IgM.

Invalid Result

The test should be considered invalid if :

1. No band/s appear in result window of Malaria Pv/Pf, *S.typhi* IgM and Dengue NS 1 test.
2. Malaria Pv/Pf : If only test bands (T1 and/or T2) appear and no control band appears.
3. *S.typhi* IgM & Dengue NS 1 : If only test band appears and no control band appears.

PERFORMANCE CHARACTERISTICS

Internal Evaluation

In an in-house evaluation study, the performance of **PyroCHECK**™ (Device) was evaluated as follows:

(I) Microscopy confirmed *P.vivax* and *P.falciparum* malaria positive whole blood specimens

Specimen Data	Nos. Tested	PyroCHECK™ (Device)			PyroCHECK™ (Device)	
		Pv Positive	Pf Positive	Negative	Specificity (95% Confidence Interval)	Sensitivity (95% Confidence Interval)
P.vivax positive	25	25	0	0	-	100% (86.28% to 100.00%)
P.falciparum positive	25	0	25	0	-	100% (86.28% to 100.00%)
Malaria Negative	100	0	0	100	100% (96.38% to 100.00%)	-

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(ii) WIDAL confirmed positive and negative whole blood specimens

Specimen Data	Nos. Tested	PyroCHECK™ (Device)			
		S.typhi IgM Positive	Negative	Specificity (95% Confidence Interval)	Sensitivity (95% Confidence Interval)
WIDAL Positive	30	30	0	-	100% (88.43% to 100.00%)
WIDAL Negative	100	03	97	97% (91.48% to 99.38%)	-

(iii) ELISA confirmed Dengue NS1 antigen positive and negative whole blood specimens

Specimen Data	Nos. Tested	PyroCHECK™ (Device)			
		NS 1 Positive	Negative	Specificity (95% Confidence Interval)	Sensitivity (95% Confidence Interval)
NS1 Positive	20	20	0	-	100% (83.16% to 100.00%)
NS 1 Negative	100	0	100	100% (96.38% to 100.00%)	-

Based on above data :
Specificity of **PyroCHECK™ (Device) : 97%** (91.48% to 99.38%)
Sensitivity of **PyroCHECK™ (Device) : 100%** (92.89% to 100.00%)

External Evaluation

In a NABL accredited reputed reference laboratory in India, **PyroCHECK™ (Device)** was tested with 90 nos. whole blood specimens of individuals with fever and 68 nos. whole blood specimens of Healthy individuals in comparison with other licensed commercial kits for Malaria Pv/Pf antigen, *S.typhi* IgM antibodies and Dengue NS 1 antigen respectively.
Results were obtained as below:

Specimens	Nos. Tested	PyroCHECK™ (Device)	Commercial kit results
P.vivax Malaria Positive	08	08	08
P.falciparum Malaria Positive	02	02	02
S.typhi IgM Positive	31	29	31
Dengue NS1 antigen Positive	49	48	49
Negative	68	67	66

Based on this evaluation:
Specificity of **PyroCHECK™ (Device) : 98.53%** (92.08% to 99.96%)
Sensitivity of **PyroCHECK™ (Device)** for **Malaria Pv & Pf antigen: 100%** (69.15% to 100.00%)
Sensitivity of **PyroCHECK™ (Device)** for ***S.typhi* IgM antibodies : 93.55%** (78.58% to 99.21%)
Sensitivity of **PyroCHECK™ (Device)** for **Dengue NS 1 antigen : 97.96%** (89.15% to 99.95%)

LIMITATIONS OF THE TEST

1. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test but should rather be made by a clinician after all clinical findings have been evaluated.
2. **PyroCHECK™** detects the presence or absence of Malaria Pv/Pf/Typhoid/Dengue infection in the whole blood specimen. It should not be used as sole criteria for the diagnosis of dengue infection.
3. Various studies have reported interference due to presence of heterophile antibodies in patient's sample. **PyroCHECK™** uses HETEROPHILIC BLOCKING REAGENT (HBR) to inhibit majority of this interference.
4. **PyroCHECK™** should be used as a screening test in clinically suspected cases only, and its results should be confirmed by other supplemental method before taking clinical decisions.
5. In some studies, it has been reported that low titre IgM antibodies to *S. typhi* may persist for about 4 months post infection. Therefore, in endemic area, samples positive yet with low signal intensity should be interpreted with caution, preferably in light of patient history. The following chart would explain the IgM sero response in *S. typhi* infected subjects after onset of fever.

Detectable IgM Response	
Onset of fever	**Percent positive**
4-6 days	43.50 %
6-9 days	92.90 %
>9 days	100 %

A negative result, i.e., the absence of detectable IgM antibody does not rule out recent or current infection, as the positivity is influenced by the time elapsed from the onset of fever and immunocompetence of the patient. However, if *S. typhi* infection is still suspected, obtain a second specimen 5-7 days later and repeat the test. Specific IgG may compete with the IgM for sites and may result in a false negative. Conversely, high titer Rheumatoid factor may result in a false positive reaction.

6. A low extent of cross reactivity may be observed with *S. paratyphi* infection.
7. **PyroCHECK™** 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*.

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8. In case of infection with *P. vivax* usually, the 'Pv' bands can be employed for monitoring success of 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. 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.
9. 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 package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

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13. Data on file: Zephyr Biomedicals.

SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Do not reuse	Do not use if package is damaged
Manufacturer	IVD *In vitro* Diagnostic Medical Device	DEVICE Device	Xn NaN3 R22, S23-46-61
Use by	REF Catalogue Number	BUF Sample Running Buffer	
Contains sufficient for <n> tests	LOT Batch Number / Lot Number	PIPETTE Disposable Plastic Specimen Applicator	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.
Date of Manufacture	This side up		

Manufactured by:

Zephyr Biomedicals

A Division of Tulip Diagnostics (P) Ltd.

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