WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT

Product: ONE STEP Malaria (Pf) Test
WHO reference number: PQDx 0626-017-00

ONE STEP Malaria (Pf) Test with product codes ITPW11007-TC25 and ITPW11007-TC40 manufactured by InTec Products, Inc., Rest of World regulatory, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 28 May 2024.

Summary of WHO Prequalification Assessment for One Step Malaria (Pf) Test

	Date	Outcome
Prequalification listing	28 May 2024	listed
Dossier assessment	12 April 2024	MR
Site inspection of quality management system	11 to 13 October 2023	MR
Product performance evaluation	Quarter 3 2021	MR

MR: Meets Requirements

Intended use

According to the intended use claim from InTec Products, Inc., *"ONE STEP Malaria (Pf) Test is a colloidal gold, two site sandwich immunoassay utilizing whole blood (venous and fingerstick) for the detection of Pf specific histidine rich protein-II (Pf HRP-II). This rapid test can be used as an aid in the diagnosis of malarial infection caused by P .falciparum for symptomatic patients, including adults (including pregnant women) and children. This test is intended for professional use by laboratory professionals, trained healthcare workers or trained lay providers in laboratory and non-laboratory settings."*

Assay description

According to the claim of assay description from InTec Products, Inc, "*Colloid gold conjugated-Anti-HRP-II(a) is pre-applied in the reaction pad. Anti-HRP-II(b) is pre-coated in the Pf band region of the membrane. After the buffer is added, red blood cells will be lysed. For Pf positive specimens, colloid gold conjugated-Anti-HRP-II(a) will react with HRP-II released from red blood cells and form a colloid gold conjugated-Anti-HRP-II(a)-HRP-II complex. The complex will migrate through the test strip and be captured by Anti-HRP-II(b) pre-coated in the Pf band region, forming a Pf band. A negative specimen will not produce a Pf band due to the absence of colloid gold conjugated-Anti-HRP-II(a)-HRP-II complex. To ensure assay validity, a control band in the control band region will appear at the end of the*

test procedure regardless of the test result. Only when the control band appears the assay is valid."

Test kit contents

Component	25 Tests/Kit (T/K) (ITPW11007-TC25)	40 T/K (ITPW11007-TC40)
Cassette	1 x 25 pieces	1 x 40 pieces
Dropper	1 x 25 pieces	1 x 40 pieces
Buffer bottle	2mL x 3 bottles	2mL x 4 bottles
Lancet	1 x 25 pieces	1 x 40 pieces
Alcohol swab	1 x 25 pieces	1 x 40 pieces
Instructions for use	1 x 1 piece	1 x 1 piece

Items required but not provided:

- Timer or stopwatch.
- Blood sampling tools (sterile gauze pad, venous puncture device, collection tube with EDTA/heparin sodium/sodium citrate for whole blood or plasma, collection tube with no anticoagulant for serum.)
- Biohazard waste bin and sharps bin.
- Disposable gloves.
- A calibrated precision pipette and applicable pipette tips.

Storage

The test kit should be stored at 2-40 °C.

Shelf-life upon manufacture

24 months.

Warnings/limitations

Please refer to the current version of the manufacturer's instructions for use attached to this public report.

Prioritization for Prequalification Assessment

Based on the established criteria, the ONE STEP Malaria (Pf) Test was given priority for the WHO prequalification assessment.

Dossier assessment

InTec Products, Inc. submitted a product dossier for One Step Malaria (Pf) Test as per the "Instructions for compilation of a product dossier" (PQDx_018). The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external technical experts (assessors) appointed by WHO. The manufacturer's responses to the nonconformities found during dossier screening and assessment findings were accepted on 12 April 2024.

Commitments for prequalification

1. The manufacturer must submit additional information regarding the formulation and composition. At the time of publication of this Public Report, the information was provided and is under review.
2. The manufacturer must submit additional information regarding the specimen type. At the time of publication of this Public Report, the information was provided and is under review.
3. The manufacturer must submit additional information regarding analytical specificity. At the time of publication of this Public Report, the information was provided and is under review.
4. The manufacturer must submit additional information regarding the clinical evaluation. At the time of publication of this Public Report, the information was provided and is under review.

Based on the product dossier screening and assessment findings, the product dossier for ONE STEP Malaria (Pf) Test Test meets WHO prequalification requirements.

Manufacturing site inspection

An onsite inspection of InTec Products, Inc. at 332 Xinguang Rd, Xinyang IND AREA, Haicang, Xiamen 361011, China, was conducted from 11 to 13 October 2023. At the time of considering the product application for Prequalification, the Manufacturer of the product had a well-established quality management system and manufacturing practices in place that would support the manufacture of a product of consistent quality. Routine inspections of the Manufacturing site will be conducted with copies of the WHO Public Inspection Report (WHOPIR) published on the WHO Prequalification web page as per Resolution WHA57.14 of the World Health Assembly. Note that a WHOPIR reflects the information on the most current assessment performed at a manufacturing site for in vitro diagnostic products and summarises the assessment findings.

https://extranet.who.int/pqweb/vitro-diagnostics/who-public-inspection-reports

All published WHOPIRs are with the agreement of the manufacturer.

The onsite inspection was accepted on 23 February 2024.

Based on the site inspection and corrective action plan review, the quality management system for the ONE STEP Malaria (Pf) Test meets WHO prequalification requirements.

Product performance evaluation

ONE STEP Malaria (Pf) Test was evaluated in the 3rd quarter of 2021 at the Centers for Disease Control and Prevention on behalf of WHO according to protocol PQDx_317, version 2.1.

ONE STEP Malaria (Pf) Test was evaluated against a *Plasmodium falciparum* cultured line panel, *P. falciparum* wild-type parasite panel, P. vivax wild-type parasite panel and a *P. falciparum* and *P.vivax* negative panel.

Performance characteristics		
	P. falciparum	***P. vivax***
Panel detection score at 200 parasites/µL (N=100)	89/100, 89.0%	*NA for Pf assays*
False positive results %	Negative specimens: 0/200, 0% Of which, clean negative specimens: 0/104, 0.0% *P. vivax* specimens at 200 and 2000 parasites/µL: 0/210, 0%	
Invalid rate % (N= 1010)	0/1010, 0.0%	
Inter-reader variability % (N= 1010)	20/1010, 2.0%	
The lowest concentration of HRP-2/pLDH was detected using the 1st WHO International standard for Pf antigens (NIBSC code: 16/376)	31.3 IU/mL on both lots	

Operational characteristics and ease of use

This assay does not require laboratory equipment and can be performed in laboratories with limited facilities or in non-laboratory settings.
The assay was found easy to use by the operators performing the evaluation.

Key operational characteristics	
Specimen types and volume	5 µL of capillary or venous whole blood
Number of steps*	2 steps in total 1 step with specimen transfer device (precision pipette was used during the evaluation)
Time to result	15 minutes
Endpoint stability (interval)	5 minutes (the test can be read between 15 and 20 minutes after the addition of diluent)
Internal QC	Yes, reagent addition control

** Definition: each action required to obtain a result (excluding specimen collection, device preparation – opening the pouch), e.g. for RDTs: add specimen, add buffer (2 steps).*

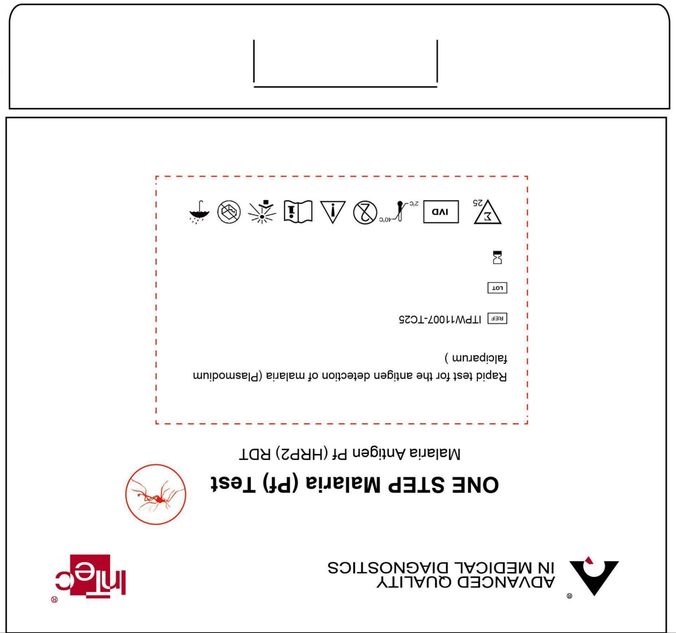
Based on these results, the performance evaluation for the ONE STEP Malaria (Pf) Test meets the WHO prequalification requirements.

Labelling

1. **Labels**
2. **Instructions for use**

1. Labels

1.1. 25 T/K, product code ITPW11007-TC25



ONE STEP Malaria (Pf) Test

Malaria Antigen Pf (HRP2) RDT

Malaria

Content
25 Cassettes
25 Droppers
3 Buffer bottles
25 Lancets
25 Alcohol swabs
1 Instructions for use

Required but not provided
Timer or stopwatch
Blood sampling tools
Biohazard waste bin
Sharps bin
Disposable gloves

REF ITPW11007-TC25

IVD

REF ITPW11007-TC25

ADVANCED QUALITY
IN MEDICAL DIAGNOSTICS

Content
25 Cassettes
25 Droppers
3 Buffer bottles
25 Lancets
25 Alcohol swabs
1 Instructions for use

Required but not provided
Timer or stopwatch
Blood sampling tools
Biohazard waste bin
Sharps bin
Disposable gloves

ONE STEP Malaria (Pf) Test

Malaria Antigen Pf (HRP2) RDT

InTec PRODUCTS, INC.
332 Xinguang Road, Xinyang Industrial Area,
Haicang, 361022, Xiamen, Fujian,P.R.China
Tel: +86 592 6807188
Website: www.intecasi.com
Email: intecproducts@asintec.com

ADVANCED QUALITY
IN MEDICAL DIAGNOSTICS

Malaria

InTec PRODUCTS, INC.

ADVANCED QUALITY
IN MEDICAL DIAGNOSTICS

InTec PRODUCTS, INC.
332 Xinguang Road, Xinyang Industrial Area,
Haicang, 361022, Xiamen, Fujian,P.R.China

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Website: www.intecasi.com
Email: intecproducts@asintec.com

01.05.11.087-240402

One Step Malaria (Pf) Test

Malaria Antigen Pf (HRP2) RDT

Malaria

ADVANCED QUALITY
IN MEDICAL DIAGNOSTICS

One Step Malaria (Pf) Test

REF

LOT

Contents

1 Cassette

1 Dropper

1 Desiccant

InTec PRODUCTS, INC.
332 Xinguang Road, Xinyang Industrial Area,
Haicang, 361022, Xiamen, Fujian,P.R.China
Tel: +86 592 6807188
Website: www.intecasi.com
Email: intecproducts@asintec.com

IVD

2℃ 40℃

Buffer bottle

For One Step Malaria (Pf) Test

IVD

REF ITPW11007-TC25

Vol: 2mL

LOT

Storage: 2-40℃

Alcohol Swabs

Produce Code：06-023060
Specifications of Blade：30mm × 60mm
Qty: 25Pcs/Bag

Manufacturer:
SteriLance Medical (Suzhou) Inc .
No.168 PuTuoShan Road, New District,
215153 Suzhou,Jiangsu, P.R.China

6 945630 100000

REF 06-023060

LOT XXXXX

YYYY-MM-DD

YYYY-MM-DD

Intended use： It's used for skin disinfection before blood sampling, injection and infusion.

Instructions for Use：
1. Open the package and take out the alcohol swab;
2. Use the alcohol swab to wipe the skin;
3. After the alcohol on the surface is dried, the disinfection is completed;
4. Discard the used alcohol swab in the special container.

Contraindications：
1. Do not use if there is skin infection or skin damage on the wiping part.
2. It should be used with caution or follow the doctor's advice for the user allergic to alcohol.

Caution：
• This product is not suitable for trauma or burns. Stop the use and consult your doctor if you have symptoms of redness, allergies, swelling, pain or inflammation.
• Do not use the product if the package of alcohol swab is damaged.
• Alcohol swab is for external use only, not for eyes or mucous membrane. Please consult your doctor if it touches the wound.
• Do not use if there is skin infection or skin damage on the wiping part.
• The alcohol swab is for single use and shall be kept away from naked fire.
• Keep the alcohol swab away from children.
• Do not use beyond the use-by date.

Symbolic interpretation:

Do not re-use | Caution | Catalogue number | Do not use if package is damaged | Manufacturer

Batch code | Date of manufacture | Use-by date

PANTONE Reflex Blue C

Size:50*40mm

Blood Lancets

Model / Specification : I / 28G

LOT LOT NO. : 240607001

EXP. DATE : 2029-06-06

MFG. DATE : 2024-06-07

QTY : 25pcs

(01)06949517006997(17)290606
(11)240607(10)240607001

STERILE MD

STERILE R -20°C 40°C 0% 80% UDI

CE 0197

EC REP **Linkfar Healthcare GmbH**
Niederrheinstraße 71, 40474 Düsseldorf, Germany
TEL: +49-21130036618

Shandong Lianfa Medical Plastic Products Co., Ltd.
No.15huangshan Sanjian Road, Zhangqiu, Jinan City, 250200, Shandong P. R. China

1.2. 40 T/K, product code ITPW11007-TC40

ONE STEP Malaria (Pf) Test

Malaria Antigen Pf (HRP2) RDT

Malaria

Content
40 Cassettes
40 Droppers
4 Buffer bottles
40 Lancets
40 Alcohol swabs
1 Instructions for use

Required but not provided
Timer or stopwatch
Blood sampling tools
Biohazard waste bin
Sharps bin
Disposable gloves

REF ITPW11007-TC40

Σ 40 | IVD | 2°C-40°C

REF ITPW11007-TC40

Content
40 Cassettes
40 Droppers
4 Buffer bottles
40 Lancets
40 Alcohol swabs
1 Instructions for use

Required but not provided
Timer or stopwatch
Blood sampling tools
Biohazard waste bin
Sharps bin
Disposable gloves

ONE STEP Malaria (Pf) Test
Malaria Antigen Pf (HRP2) RDT

InTec PRODUCTS, INC.
332 Xinguang Road, Xinyang Industrial Area,
Haicang, 361022, Xiamen, Fujian,P.R.China
Tel: +86 592 6807188
Website: www.intecasi.com
Email: intecproducts@asintec.com

ADVANCED QUALITY
IN MEDICAL DIAGNOSTICS

Malaria

InTec PRODUCTS, INC.

ADVANCED QUALITY
IN MEDICAL DIAGNOSTICS

InTec PRODUCTS, INC.
332 Xinguang Road, Xinyang Industrial Area,
Haicang, 361022, Xiamen, Fujian,P.R.China

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Email: intecproducts@asintec.com

ADVANCED QUALITY
IN MEDICAL DIAGNOSTICS

ONE STEP Malaria (Pf) Test

Malaria Antigen Pf (HRP2) RDT

Rapid test for the antigen detection of malaria (Plasmodium falciparum)

REF ITPW11007-TC40

LOT

Σ 40 | IVD | 2°C-40°C

01.05.11.091-240402

One Step Malaria (Pf) Test

Malaria Antigen Pf (HRP2) RDT

Malaria

ADVANCED QUALITY
IN MEDICAL DIAGNOSTICS

One Step Malaria (Pf) Test

REF

LOT

Contents

1 Cassette

1 Dropper

1 Desiccant

InTec PRODUCTS, INC.
332 Xinguang Road, Xinyang Industrial Area,
Haicang, 361022, Xiamen, Fujian,P.R.China
Tel: +86 592 6807188
Website: www.intecasi.com
Email: intecproducts@asintec.com

IVD

2℃ 40℃

Buffer bottle

For One Step Malaria (Pf) Test

IVD

REF ITPW11007-TC40

Vol: 2mL

LOT

Storage: 2-40℃

Alcohol Swabs

Produce Code：06-023060
Specifications of Blade：30mm × 60mm
Qty: 40Pcs/Bag

Manufacturer:
SteriLance Medical (Suzhou) Inc .
No.168 PuTuoShan Road, New District,
215153 Suzhou,Jiangsu, P.R.China

6 945630 100000

REF 06-023060

LOT XXXXX

YYYY-MM-DD

YYYY-MM-DD

Intended use： It's used for skin disinfection before blood sampling, injection and infusion.

Instructions for Use：
1. Open the package and take out the alcohol swab;
2. Use the alcohol swab to wipe the skin;
3. After the alcohol on the surface is dried, the disinfection is completed;
4. Discard the used alcohol swab in the special container.

Contraindications：
1. Do not use if there is skin infection or skin damage on the wiping part.
2. It should be used with caution or follow the doctor's advice for the user allergic to alcohol.

Caution：
• This product is not suitable for trauma or burns. Stop the use and consult your doctor if you have symptoms of redness, allergies, swelling, pain or inflammation.
• Do not use the product if the package of alcohol swab is damaged.
• Alcohol swab is for external use only, not for eyes or mucous membrane. Please consult your doctor if it touches the wound.
• Do not use if there is skin infection or skin damage on the wiping part.
• The alcohol swab is for single use and shall be kept away from naked fire.
• Keep the alcohol swab away from children.
• Do not use beyond the use-by date.

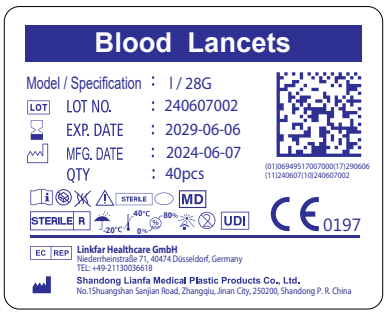
Symbolic interpretation:

Do not re-use | Caution | Catalogue number | Do not use if package is damaged | Manufacturer

Batch code | Date of manufacture | Use-by date

PANTONE Reflex Blue C

Size:50*40mm



2. Instructions for use[1]

[1] English version of the IFU was the one that was assessed by WHO. The manufacturer is responsible for ensuring correct translation into other languages.

InTec PRODUCTS, INC.

REF ITPW11007-TC25
ITPW11007-TC40
01.05.14.078-240507

One Step Malaria (Pf) Test

FOR IN VITRO DIAGNOSTIC USE ONLY. IVD
Please read this instructions for use carefully prior to use and strictly follow the instructions.
Reliability of the assay cannot be guaranteed if there are any deviations from the instructions for use.

Clinical significance

Malaria is a serious, sometimes fatal, parasitic disease, widespread in the tropical and subtropical regions (mainly Africa, South America, and Southeast Asia). It is characterized by fever with chills, anemia and is caused by Plasmodium parasite that is transmitted to people through the bites of infected female Anopheles mosquitoes.
Among several parasitic Plasmodium species that cause malaria in humans (i.e., P. falciparum, P. vivax, P. malariae, P. ovale, and P. knowlesi), P. falciparum (Pf) and P. vivax (Pv) are two pathogens posing the greatest threat as they are the deadliest or most prevalent.

Intended use

One Step Malaria (Pf) Test is a colloidal gold, two site sandwich immunoassay utilizing whole blood (venous and fingerstick) for the detection of Pf specific histidine rich protein-II (Pf HRP-II). This rapid test can be used as an aid in the diagnosis of malarial infection caused by P .falciparum for symptomatic patients[1], including adult (including pregnant women) and children. This test is intended for professional use by laboratory professionals, trained healthcare workers or trained lay providers in laboratory and non-laboratory settings.

Summary

One Step Malaria (Pf) Test is based on immunochromatography, and used for Pf HRP-II detection in human whole blood (venous and fingerstick)[2-3]. This is a simple, visual qualitative test that detects Pf HRP-II in humanwhole blood, and presents the result within 20 minutes[3].

Test principle[4]

Colloid gold conjugated-Anti-HRP-II(a) is pre-applied in the reaction pad. Anti-HRP-II(b) is pre-coated in the Pf band region of the membrane. After the buffer is added, red blood cells will be lysed.
For Pf positive specimens, colloid gold conjugated-Anti-HRP-II(a) will react with HRP-II released from red blood cells and form a colloid gold conjugated-Anti-HRP-II(a)-HRP-II complex. The complex will migrate through the test strip and be captured by Anti-HRP-II(b) pre-coated in the Pf band region, forming a Pf band. A negative specimen will not produce a Pf band due to the absence of colloid gold conjugated-Anti-HRP-II(a)-HRP-II complex. To ensure assay validity, a control band in the control band region will appear at the end of the test procedure regardless of the test result.
Only when the control band appears the assay is valid.

Storage conditions and Stability

One Step Malaria (Pf) Test shall be stored at 2-40°C. Test cassette should be used immediately upon opening the foil pouch. Buffer should be used within 8 weeks after opening.

⚠ Warnings and precautions

The warnings and precautions are included, but not limited to the following:

- This product is for in vitro diagnosis of the infection of Pf only, and other diseases cannot be analyzed with any component of this kit.
- Wear gloves during the entire testing process.
- Do not use expired reagents or test cassettes.
- Do not use accessories if the seal or package is broken.
- Do not use test cassette if the foil pouch is damaged or the seal is broken.
- Do not use the provided lancet if the cap is already pulled off before use.
- Do not reuse the accessories. All the accessories are for single use.
- Do not reuse the test cassette. Each cassette enclosed in a foil pouch is only for single use.
- Do not suck buffer or specimen by mouth.
- Do not eat or smoke while handling specimens.
- Do not store specimen in dropper, it is only used for specimen collection.
- Do not use pooled specimens or specimens other than specified (i.e. saliva, urine).
- Do not interchange reagents among kits of different batch number or even products.
- Do not perform the test under environment which leads to rapid evaporation (e.g. close to a running fan or air conditioner).
- Ensure the specimen is added correctly prior to the addition of buffer.
- Avoid contact between the "S" well of cassette and buffer bottle to prevent contamination of buffer.
- Clean and disinfect all the areas that may be contaminated by spills of specimens or reagents with appropriate disinfectant.
- Decontaminate and dispose of all specimens, reagents, accessories and other potentially contaminated materials as infectious wastes in a biohazard container. Used lancet should be disposed of in a sharps bin.
- Operate the test at conditions that are both >40°C and >70% relative humidity (RH) might lead to incorrect results.
- Sample buffer contains 0.3% Triton X-100 and 0.01% sodium azide. If contact with buffer to the eyes and/or skin, wash affected area with soap and water immediately. Do not dispose buffer enter drains, and offer surplus solutions to a licensed disposal company (dispose of contents/container to an approved waste disposal plant).

Reagents and materials provided

Table 1 Reagents and materials provided

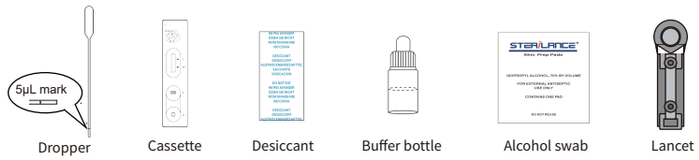
Components	25 tests (ITPW11007-TC25)	40 tests (ITPW11007-TC40)
Cassette	1 x 25 pieces	1 x 40 pieces
Dropper	1 x 25 pieces	1 x 40 pieces
Buffer bottle	2mL x 3 bottles	2mL x 4 bottles
Lancet	1 x 25 pieces	1 x 40 pieces
Alcohol swab	1 x 25 pieces	1 x 40 pieces
Instructions for use	1 x 1 piece	1 x 1 piece

Materials required but not provided

- Timer or stopwatch
- Blood sampling tools (sterile gauze pad, venous puncture device, collection tube with EDTA/heparin sodium/sodium citrate for whole blood.)
- Biohazard waste bin and sharps bin
- Disposable gloves
- A calibrated precision pipette and applicable pipette tips

Preparation

1. Unseal the foil pouches. The components are as below:

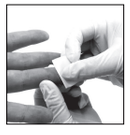


2. Wear gloves.

3. Mark the sample ID number.

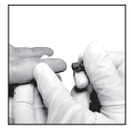
I. Fingerstick whole blood

4. Clean the finger with alcohol swab and leave it to dry.



5. Twist the lancet cap for over 180° and remove it.

6. Place the lancet firmly on side of finger (avoid callus) to trigger it.



7. Gently press around the bleeding point. Wipe away the first drop of blood.

8. Gently squeeze and release the bulb of the dropper to collect blood until reaching 5μL mark.

9. Touch the dropper tip on the pad of "**S**" wellto add all the blood collected.

10. Add **3 drops** of buffer into "**D**" well immediately.

11. Wait and interpret the result between 15-20 minutes.

II. Venous whole blood

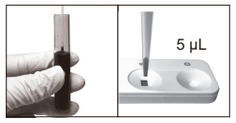
4. Gently squeeze and release the bulb of the dropper to collect blood until reaching 5μL mark. Touch the dropper tip on the pad of "**S**" well, to add all the blood collected.

Or, if using transfer pipette add 5 μL whole blood into "**S**" well.

Dropper

or

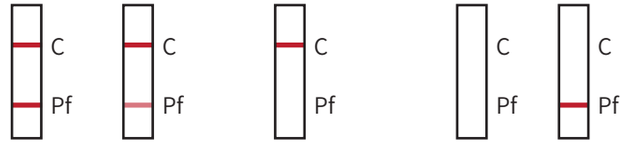
Pipette



5. Add **3 drops** of buffer into "**D**" well immediately.

6. Wait and interpret the result between 15-20 minutes.

Test results



Positive results | Negative result | Invalid results

Specimen collection and storage

Fingerstick whole blood

1) Rub the target finger (middle or ring finger) to stimulate blood flow, and avoid calloused areas of the finger. Clean the finger with an alcohol swab (Figure I.4) and leave it to dry.
2) Prick the finger with the provided lancet: (a)Twist clockwise the protective cap and remove it, see Figure I.5 for details. (b) Place the lancet firmly on the finger to trigger it, see Figure I.6 for details.
3) Gently press the bleeding point (avoid excessive bleeding). Wipe away the first drop of blood with a sterile gauze pad (Figure I.7). Allow a new drop of blood to form.
4) Transfer the blood specimen with the dropper provided. Gently squeeze the bulb of the dropper, touch the blood, and gently release the bulb to draw up the blood (Figure I.8).

Note: The fingerstick blood should be tested immediately after collection.

Venous whole blood

Collect whole blood specimen into a collection tube (with specified anticoagulant, namely EDTA, heparin sodium or sodium citrate) according to standard venous blood sampling process. Then gently mix the venous blood collection tube by inverting the specimen to make it homogenous for sampling. Other anticoagulants may lead to incorrect results.

Notes:

- Venous whole blood specimens can be stored at 2-8°C for up to 7 days if not tested immediately. Store at -18°C or below for 30 months. Frozen specimens shall be equilibrated to room temperature (10-30°C) before testing. Multiple freeze-thaw cycles should be avoided (3 times at most).
- Avoid using hyperlipidemia or excessively aged specimens.

Test procedure

1. Do not open the pouch until ready to perform a test. Use the test under low environment humidity (RH≤ 70%) within 1 hour.
2. Equilibrate all reagents and specimens to room temperature (10-30°C) before use.
3. Unseal the foil pouch and put the cassette on a flat, clean and dry surface.
4. Mark the sample ID number on test cassette.
5. Gently squeeze and release the bulb of the dropper to collect blood until reaching 5μL mark (or 5μL by the transfer pipette) and touch the dropper tip on the pad of "S" well to add all the blood collected.
6. Then add 3 drops of lysis buffer into "D" well and start the timer immediately.
7. Wait for at least 15 minutes (and 20 minutes at most) to interpret the result.

⚠ Caution:

- Always apply specimen with a new and clean dropper or pipette tip to avoid cross contamination.
- Negative results cannot rule out the possibility of the exposure to or the infection with of Pf.

Result interpretation

Positive: Purplish red bands appear at both the Pf band area (even though very faint) and the control band area indicates a positive result.
Negative: Purplish red band only appears on control band region indicates a negative result.
Invalid 1: A purplish red band appears only at the Pf band area of the cassette. Repeat the test. Contact the supplier if the control band remains invisible.
Invalid 2: Purplish red band appears at neither the Pf band area nor the control band area of the cassette. Repeat the test. Contact the supplier if the control band remains invisible.

Performance characteristics

The performance characteristics of One Step Malaria (Pf) Test was established based on external clinical evaluations and internal studies. Concluded from the study results, the analytical performance and clinical performance were summarized as below:

1. Potential interfering substances

The following listed 25 potentially interfering substances have no impact on the test result of One Step Malaria (Pf) Test. Although no interference observed in the study, the possibility cannot be excluded completely.

No.	Type of Specimen	Potential Interfering Substance	Sample Quantity
1	Endogenous Substance	Total protein	3
2		Hemoglobin	1
3		Anti-Escherichia coli (AEC)	3
4		(Nonspecific) IgM	3
5		(Nonspecific) IgG	3
6		Human anti-mouse antibody (HAMA)	1
7		Bilirubin	1
8		Rheumatoid factors (RF)	3
9		Anti-nuclear antibodies (ANA)	3
10		Systemic Lupus Erythematosus (SLE)	13

11	Endogenous Substance	Triglyceride	13
12		Cholesterol	10
13		Glucose	10
14		Uric acid	5
15		Multiple blood transfusions	5
16		Pregnant women (multifarious)	20
17	Exogenous Substance	Antiparasitic	1
18		Antimalarial	1
19		Antiretroviral	1
20		Anti-tuberculosis	1
21		Aspirin	1
22		Paracetamol	1
23		Ibuprofen	1
24		Alcohol	1
25		Caffeine	1

2.Potential cross-reacting substances

The following listed 70 potentially cross-reacting substances have no impact on the test result of One Step Malaria (Pf) Test. Although no cross reactivity occurred in the study, the possibility cannot be completely excluded.

No.	Potential Cross-reacting Pathogen	Sample Quantity	No.	Potential Cross-reacting Pathogen	Sample Quantity
1	Hepatitis A virus (HAV)	3	36	Cryptococcus neoformans	1
2	Hepatitis C virus (HCV)	30	37	Rothia mucilaginosa	1
3	Hepatitis B virus (HBV)	26	38	Haemophilus influenzae	1
4	Human immunodeficiency virus (HIV)	14	39	Aspergillus fumigatus	1
5	Treponema pallidum (TP)	20	40	Aspergillus flavus	1
6	Influenza A (Flu A)	23	41	Streptococcus oralis	1
7	Influenza B (Flu B)	21	42	Streptococcus salivarius	1
8	Mycoplasmal pneumonia (MP)	3	43	Adenovirus type 1	1
9	Dengue	8	44	Adenovirus type 2	1
10	Mycobacterium tuberculosis (TB)	22	45	Adenovirus type 3	1
11	Toxoplasma (Toxo)	5	46	Adenovirus type 4	1
12	Measles virus	1	47	Adenovirus type 5	1
13	Varicella-zoster virus (VZV)	1	48	Adenovirus type 7	1
14	Epstein-Barr virus (EBV)	1	49	Adenovirus type 55	1
15	Mumps virus	1	50	Rhinovirus type B70	1
16	Streptococcus pneumonia type 14	1	51	Rhinovirus type A2	1
17	Staphylococcus aureus	1	52	Respiratory syncytial virus type A	1
18	Candida albicans	1	53	Respiratory syncytial virus type B	1
19	Human metapneumovirus	1	54	Herpes simplex virus	1
20	Bordetella pertussis	1	55	SARS coronavirus	10
21	Neisseria meningitidis	1	56	MERS coronavirus	1
22	Human cytomegalovirus	1	57	Plasmodium vivax (Pv)	6
23	Norovirus	1	58	Schistosomiasis	2
24	Bocavirus	1	59	Leishmania	9
25	Chlamydia pneumoniae	1	60	Plasmodium malariae (Pm)	8
26	Klebsiella pneumoniae	1	61	Plasmodium ovale (Po)	3
27	Neisseria gonorrhoeae	1	62	Rheumatoid factors	5
28	Pyogenic streptococcus	1	63	Anti-nuclear antibodies	5
29	Corynebacterium ulcerans	1	64	Upper respiratory track infection (URTL)	14
30	Legionella pneumophila	1	65	Respiratory track infection (UTI)	8
31	Staphylococcus epidermidis	1	66	Tonsilitis	1
32	Lactobacillus casei	1	67	Dental caries	1
33	Moraxella catarrhalis	1	68	Diarrhoea	3
34	Escherichia coli	1	69	Enteric fever	2
35	Pseudomonas aeruginosa	1	70	Pneumonia	3

3. Hook effect

The hook effect was evaluated by testing dilution series prepared by clinical samples of high parasite density up to 113,820 parasites/μL for Pf, whereas high antigen concentration up to 68,496.720 ng/mL for Pf. No hook effect was observed in the test results. Still, hook effect cannot be completely excluded especially when parasite density/antigen concentration is higher than the above values.

4. Diagnostic sensitivity & specificity

External clinical evaluations were conducted in three different sites located in Africa, Asia. Overall, a total of 1236 clinical samples (fingerstick/venous whole blood) were tested by One Step Malaria (Pf) Test, with samples characterized by composite reference method, first by microscopy and further confirmed by PCR. Among these samples, 533 were malaria Pf positive, 703 malaria negative. The diagnostic sensitivity and specificity achieved from the studies are listed in tables below.

After 1 invalid sample results were excluded from the final analysis:

Clinical sensitivity for Malaria Pf: 96.06% (95% CI: 94.05%-97.41%)

Clinical specificity for Malaria Pf: 98.43% (95% CI: 97.22%-99.12%)

Table 2 Diagnostic Sensitivity study in three sites

Study site	Number of specimens reactive by PCR and microscopy	Number of invalid tested	Number of specimens reactive by InTec RDT	Number of specimens falsely-non-reactive	Sensitivity	95% CI
Fingerstick whole blood						
Tanzania	383	0	364	19	95.04%	92.38%-96.80%
Venous whole blood						
Bangladesh	100	0	98	2	98.00%	93.00%-99.45%
China	50	0	50	0	100.00%	92.87%-100.00%
Based on above data:						
	Total number	Number of invalid tested	Number of specimens reactive by InTec RDT	Number of specimens falsely-non-reactive	Overall sensitivity	95% CI
Total	533	0	512	21	96.06%	94.05%-97.41%

Table 3 Diagnostic Specificity study in three sites

Study site	Number of specimens non-reactive by PCR and microscopy	Number of invalid tested	Number of specimens non-reactive by InTec RDT	Number of specimens falsely-reactive	Specificity	95% CI
Fingerstick whole blood						
Tanzania	533	1	521	11	97.93%	96.34%-98.84%
Venous whole blood						
Bangladesh	125	0	125	0	100.00%	97.02%-100.00%
China	45	0	45	0	100.00%	92.14%-100.00%
Based on above data:						
	Total number	Number of invalid tested	Number of specimens non-reactive by InTec RDT	Number of specimens falsely-reactive	Overall specificity	95% CI
Total	703	1	691	11	98.43%	97.22%-99.12%

5. Analytical sensitivity (Limit of detection)

The limit of detection for Malaria Pf is 50 parasites/μL according to the level of parasite based on calibration with reference material.

6. Blood type equivalence study

The equivalence of testing different blood types was investigated and the study results demonstrated no significant difference among fingerstick blood and venous blood preserved in common anticoagulants EDTA, heparin sodium and sodium citrate.

7. Precision

The repeatability and reproducibility of One Step Malaria (Pf) Test has been evaluated by within-run, between-run, between sites, between days, between operators and between lots studies using in-house control samples. The study results indicated 100% repeatability and 100% reproducibility.

Limitations ⚠

- The kit is designed to detect malaria Pf antigen in human whole blood. Specimens other than those specified may not supply accurate results and the device will not notify this kind of misuse to the user.
- The intensity of test band does not necessarily correlate to the titer of antigen in specimen.
- The presence of the control band only indicates the flow of the conjugate.
- When a specimen contain high concentration of Pf antigen is tested on the device, the control band could be absent due to the test principle.
- As this product is intended to detect Pf antigen from individuals, clinical diagnosis on Pf infection should also be correlated with clinical presentations and epidemiological data.
- A negative result should not exclude the possibility of infection caused by Pf. A negative result can also occur in the following circumstances:
  - Recently acquired Pf infection.
  - Low levels of antigen below the detection limit of the test.
  - Pf antigen in the patient that do not react with specific antigens utilized in the assay configuration, in exceptional cases this may lead to observation of negative results.
  - Specimens are not properly stored.
  - High concentrations of a particular analyte.
  - Recently discovered sub-strain of Pf.
  - Samples with Pf HRP-II/III gene deletions.
- For reasons above, care should be taken in interpreting negative results. Other clinical data (e.g., symptoms or risk factors) should be used in conjunction with the test results.
- Avoid using clotted or excessively viscous specimens.
- The product may give a false positive result even after the patient was treated for malaria several weeks before testing. The test cannot be used to monitor treatment response to antimalarials.

References

1. World Health Organization. Global Malaria Programme. https://www.who.int/teams/global-malaria-programme/case-management/diagnosis.
2. Quintana M, Piper R, Boling H L, et al. Malaria diagnosis by dipstick assay in a Honduran population with coendemic Plasmodium falciparum and Plasmodium vivax[J]. The American journal of tropical medicine and hygiene, 1998, 59(6): 868-871.
3. Murray C K, Bennett J W. Rapid diagnosis of malaria[J]. Interdisciplinary Perspectives on Infectious Diseases, 2009, 2009.
4. Moody A. Rapid diagnostic tests for malaria parasites[J]. Clinical microbiology reviews, 2002, 15(1): 66-78.

Symbols

⚠	CAUTION		KEEP DRY		DO NOT REUSE
	KEEP AWAY FROM SUNLIGHT		TEMPERATURE LIMITATION		CONSULT INSTRUCTIONS FOR USE
	MANUFACTURER	IVD	IN VITRO DIAGNOSTIC MEDICAL DEVICE	Σ N	CONTAINS SUFFICIENT FOR N TESTS
LOT	BATCH CODE	REF	CATALOGUE NUMBER		USE-BY DATE
	DO NOT USE IF PACKAGE IS DAMAGED				

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