CE

Truenat®

Malaria Pv/Pf

Chip-based Real Time Duplex PCR Test for *Plasmodium vivax* and *Plasmodium falciparum*

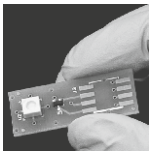
1. INTENDED USE

Truenat® Malaria Pv/Pf (REF 601010005 / 601010020 / 601010025 / 601010050 / 601010100 / 601010200) is a chip-based Real Time Polymerase Chain Reaction (PCR) test for the quantitative detection of ***Plasmodium vivax*** and ***Plasmodium falciparum*** in human blood specimen and aids in the differential diagnosis of infection with Malaria caused by *Plasmodium vivax* and *Plasmodium falciparum*. **Truenat® Malaria Pv/Pf** runs on **Truelab®** Real Time micro PCR Analyzers. **Truenat® Malaria Pv/Pf** is an *in vitro* diagnostics test meant for professional use only.

2. INTRODUCTION

Malaria is a vector borne infectious disease caused by parasitic protozoans of the genus *Plasmodium* and is transmitted by the bite of infected female *Anopheles* mosquito. Of the five *Plasmodium* species known to cause disease in humans, *P. vivax* (Pv) and *P. falciparum* (Pf) are the most prevalent and responsible for over 95% of the infections. In the human body, the parasites multiply in the liver, and then infect red blood cells. Malaria causes symptoms that typically include fever, periodic shivering, tiredness, vomiting, and headaches. In severe cases it can cause yellow skin, seizures, coma, or death. The disease is wide spread in the tropical and sub-tropical regions of the world such as sub Saharan Africa, Asia and Latin America.

Plasmodium falciparum is the most severe strain and known to cause the life threatening Falciparum malaria, the most dangerous malignant malaria that has the highest rate of complications and mortality. It also causes Cerebral malaria clinically reflected as neurological disorders such as nystagmus, conjugate gaze palsy, opisthotonus, seizures, and sometimes coma. *P. falciparum* has developed high levels of resistance to many anti-malarial drugs and treatment regimen vary from country to country.



Plasmodium vivax is the most frequent and widely distributed cause of recurring (Benign tertian) malaria. It is less virulent than *Plasmodium falciparum*, but can lead to severe disease and death due to splenomegaly (a pathologically enlarged spleen). In addition to anti-malarial drugs an adjunct therapy is necessary to prevent recurrence.

Early, accurate and differential diagnosis of *P. vivax* and *P. falciparum* malaria is necessary to initiate appropriate treatment, check transmission of the disease and prevent death.

Microscopic examination of stained blood smears is still considered as the "gold standard" for detection of malaria parasitemia. However, microscopy and antigen detecting RDTs are reported to be only 60-90% sensitive and show poor performance at low parasite loads. Nested PCR and real time PCR present higher sensitivity and specificity to malaria diagnosis compared to these methods. However, PCR or Real Time PCR tests have so far been restricted to centralized reference laboratories as they require skilled manpower and elaborate infrastructure. Also the turnaround time for results could take a few days.

The **Truelab®** Real Time micro PCR System enables decentralization and near patient diagnosis of *P. vivax* and *P. falciparum* malaria by making real time PCR technology rapid, simple, robust and user friendly and offering "sample to result" capability even at resource limited settings. This is achieved through a combination of lightweight, portable, mains / battery operated **Truelab®** Real Time micro PCR Analyzer and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and room temperature stable **Truenat®** micro PCR chips and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kits so that even the peripheral laboratories with minimal infrastructure and minimally trained technician can easily perform these tests routinely in their facilities and report PCR results in less than an hour. Moreover, with these devices PCR testing can also be initiated in the field level, on site.

Truenat® Malaria Pv/Pf is a disposable, room temperature stable, chip-based Real Time PCR test with dried $MgCl_2$ in reaction well and freeze dried PCR reagents in microtube for performing Real Time PCR test for detection and diagnosis of *Plasmodium vivax* and *Plasmodium falciparum* and runs on the **Truelab®** Real Time micro PCR Analyzer. It requires only six (6) µL of purified DNA to be added to the reaction well for the analysis. The intelligent chip also carries test and batch related information including standard values for quantitation. The **Truenat® Malaria Pv/Pf** chip also stores information of used test to prevent any accidental re-use of the test.

NOTE :Truelab®/ Truenat® / Trueprep® / Truepet® are all trademarks of Molbio Diagnostics Private Limited.

The Truelab® Real Time micro PCR Analyzer is protected by the following patents and patents granted: IN 2313/CHE/2007 (Patent No. 281573), WO2009/047804 and corresponding claims of any foreign counterpart(s) thereof.

The Truenat® micro PCR chip is protected by the following patents and patents pending: IN 2312/CHE/2007, WO 2009/047805 and corresponding claims of any foreign counterpart(s) thereof.

3. PRINCIPLE OF THE TEST

Truenat® Malaria Pv/Pf works on the principle of Real Time Polymerase Chain Reaction based on Taqman chemistry. The DNA from the patient sample is first extracted using **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep kit. The **Truenat® Malaria Pv/Pf** chip is placed on the chip tray of the **Truelab®** Real Time micro PCR Analyzer. Six (6) µL of the purified DNA is then dispensed using the provided micropipette and tip into the microtube containing freeze dried PCR reagents and allowed to stand for 30-60 seconds to get a clear solution. ⚠ No mixing by tapping, shaking or by reverse pipetting should be done. Six (6) µL of this clear solution is then pipetted out using the same pipette and tip and dispensed into the reaction well of the **Truenat® Malaria Pv/Pf** chip and the test is started. A positive amplification causes the dual labeled fluorescent probe in the **Truenat® Malaria Pv/Pf** chip to release the fluorophores in an exponential manner which is then captured by the built-in opto-electronic sensor and displayed as amplification curve on the analyzer screen, on a real time basis during the test run. The Cycle threshold (Ct) is defined as the number of amplification cycles required for the fluorescent signal to cross the threshold (i.e. exceed the background signal). Ct levels are inversely proportional to the amount of target nucleic acid in the sample (i.e. the lower the Ct level the greater is the amount of target nucleic acid in the sample). In the case of negative samples, amplification does not occur and a horizontal amplification curve is displayed on the screen during the test run. At the end of the test run, Falciparum or Vivax "DETECTED" or "NOT DETECTED" result is displayed and in positive cases, Ct values and parasite load in copies/mL is also displayed on the screen. Based on the detection of the internal positive control (IPC), the validity of the test run is also displayed. The IPC is a full process control that undergoes all the processes the specimen undergoes – from extraction to amplification thereby validating the test run from sample to result. Absence of or shift of IPC Ct beyond a pre-set range in case of negative samples invalidates the test run. While IPC will co-amplify in most positive cases also, in some specimen having a high target load, the IPC may not amplify, however the test run is still considered valid. The results can be printed using the **Truelab®** micro PCR printer or transferred to the lab computer/or any remote computer via Wifi network or 3G/GPRS network. Upto 20000 results in **Truelab® Uno Dx/ Duo/Quattro** can be stored on the analyzer for future recall and reference.

4. TARGET SELECTION

The target sequence for this kit has been taken from the erythrocyte binding protein (EBP) gene which is expressed on the surface of the merozoites. EBP is involved in the parasite's invasion of the red blood cells (RBC) by pathways that are specific to *Plasmodium vivax* & *Plasmodium falciparum*. The region selected is specific for *P.vivax* & *P. falciparum* respectively.

5. CONTENTS OF THE Truenat® Malaria Pv/Pf KIT

A. Individually sealed pouches, each containing
   1. **Truenat® Malaria Pv/Pf** micro PCR chip.
   2. Microtube with freeze dried PCR reagents.
   3. DNase & RNase free pipette tip.
   4. Desiccant pouch.

B. Package Insert.

REF	601010005	601010020	601010025	601010050	601010100	601010200
Σ	5T	20T	25T	50T	100T	200T

6. CONTENTS OF THE Trueprep® AUTO Universal Sample Pre-treatment Pack

A. Lysis Buffer.
B. Disposable transfer pipette (graduated).
C. Package Insert.

REF	60205AB05	60205AB20	60205AB25	60205AB50	60205AB100	60205AB200
Σ	5T	20T	25T	50T	100T	200T

7. STORAGE AND STABILITY

Truenat® Malaria Pv/Pf is stable for two (2) years from the date of manufacture if stored between 2-30°C. It is also stable for upto one (1) month at temperatures up to 45°C. Avoid exposure to light or elevated temperatures (above recommended levels). Do not freeze.

Trueprep® AUTO Universal Sample Pre-treatment Pack is stable for two (2) years from the date of manufacture if stored between 2-40°C. It is also stable for one (1) month at temperatures upto 45°C. Do not freeze.

8. MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

Truelab® Real Time micro PCR Workstation (REF 623010001 / 633010001 / 643010001 / 653010001) consisting of

1. **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device (REF603041001 / 603042001).
2. **Truelab® Uno Dx / Truelab® Duo / Truelab® Quattro** Real Time micro PCR

Analyzer (REF603021001 / 603022001 / 603023001).
3. **Truelab**® micro PCR Printer (REF 603050001).
4. **Truepet**® SPA fixed volume precision micropipette - 6 µl (REF 604070006).
5. **Truelab**® Microtube Stand (REF 603070001).

Also required additionally are: **Trueprep**® **AUTO** Universal Sample Pre-treatment Pack (REF60205AB05 / REF60205AB20 / REF60205AB25 / REF60205AB50 / REF60205AB100 / REF60205AB200), **Trueprep**® **AUTO** Universal Cartridge Based Sample Prep Kit (REF60203AR05 / REF60203AR25 / REF60203AR50 / REF60203AR100) or **Trueprep**® **AUTO v2** Universal Cartridge Based Sample Prep Kit (REF60207AR05 / REF60207AR25 / REF60207AR50 / REF60207AR100), **Truenat**® Positive Control Kit - Panel IV (REF 801040008), Powder free disposable gloves, waste disposal container with lid.

9. SPECIMEN PREPARATION FOR EXTRACTION WITH Trueprep® AUTO/AUTO v2

Truenat® **Malaria Pv/Pf** requires purified nucleic acids from blood specimen that are extracted using the **Trueprep**® **AUTO/AUTO v2** Universal Cartridge based Sample Prep Device and **Trueprep**® **AUTO/AUTO v2** Universal Cartridge based Sample Prep kit. Sample must be pre-treated using **Trueprep**® **AUTO** Universal Sample Pre-treatment pack. Transfer 250µl of blood specimen using the transfer pipette provided into the Lysis buffer tube provided and mix well. (Refer to the package insert of **Trueprep**® **AUTO** Universal Sample Pre-treatment Pack for further details).

Sample Storage and Transportation:

Sample Pre-treatment decontaminates the specimenand makes it ready for storage / transportation / extraction. The specimen in this form is stable for up to 3 days at 40ºC and 1 week at 30ºC.

Nucleic acid extraction: Use the entire content of lysis buffer tube containing Specimen for further procedure with the **Trueprep**® **AUTO / AUTO v2** Universal Cartridge Based Sample Prep Device and **Trueprep**® **AUTO / AUTO v2** Universal Cartridge Based Sample Prep Kit (Refer to the User Manual of **Trueprep**® **AUTO/AUTO v2** Universal Cartridge Based Sample Prep device and the package insert of **Trueprep**® **AUTO/AUTO v2** Universal Cartridge Based Sample Prep kit for details). ⚠ Dispose off the lysis buffer tube and transfer pipette after use, as per the section on "Disposal and Destruction" (Section17).

10. SAFETY PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. Bring all reagents and specimen to room temperature (20 - 30°C) before use.
3. Do not use kit beyond expiry date.
4. Carefully read the User Manuals, package inserts and Material Safety Data Sheets (MSDS) of all the components of the **Truelab**® **Real Time micro PCR System** before use.
5. All materials of human origin should be handled as though potentially infectious.
6. Do not pipette any material by mouth.
7. Do not eat, drink, smoke, apply cosmetics or handle contact lenses in the area where testing is done.
8. Use protective clothing and wear disposable gloves when handling samples and while performing sample extraction.

11. PROCEDURAL PRECAUTIONS

1. Check all packages before using the kit. Damage to the packaging does not prevent the contents of the kit from being used. However, if the outer packaging is damaged the user must confirm that individual components of the kit are intact before using them.
2. Do not perform the test in the presence of reactive vapours (e.g. from sodium hypochlorite, acids, alkalis or aldehydes) or dust.
3. While retrieving the **Truenat**® **Malaria Pv/Pf** micro PCR chip, microtube and the DNase & RNase free pipette tip from the pouch, ensure that neither bare hands nor gloves that have been used for previous tests are used.

12. PROCEDURAL LIMITATIONS

1. Optimal performance of this test requires appropriate specimen collection, handling, storage and transport to the test site.
2. Though very rare, mutations within the highly conserved regions of the target genome where the **Truenat**® assay primers and/or probe bind may result in the under-quantitation of or a failure to detect the presence of the concerned pathogen.
3. The instruments and assay procedures are designed to minimize the risk of contamination by PCR amplification products. However, it is essential to follow good laboratory practices and ensure careful adherence to the procedures specified in this package insert for avoiding nucleic acid contamination from previous amplifications, positive controls or specimens.
4. A specimen for which the **Truenat**® assay reports "Not Detected" cannot be concluded to be negative for the concerned pathogen. As with any diagnostic test, results from the **Truenat**® assay should be interpreted in the context of other clinical and laboratory findings.

13. CLEANING AND DECONTAMINATION

1. Spills of potentially infectious material should be cleaned up immediately with absorbent paper tissue and the contaminated area should be decontaminated with disinfectants such as 0.5% freshly prepared sodium hypochlorite [10 times dilution of 5% sodium hypochlorite (household bleach)] before continuing work.
2. Sodium hypochlorite should not be used on an acid-containing spill unless the spill- area is wiped dry first. Materials used to clean spills, including gloves should be disposed off as potentially bio-hazardous waste e.g. in a bio-hazard waste container.

14. TEST PROCEDURE

(Please also refer the **Truelab**® Real Time micro PCR Analyzer user manual)

1. Switch on the**Truelab**® Analyzer.
2. Select User and enter password.
3. For **Truelab**® **Uno Dx**, select the test profile for "Malaria PvPf" to be run from the Profiles Screen on the Analyzer screen. For **Truelab**® **Duo/Quattro**, select the Bay (Idle1/2) for **Duo** and (Idle1/2/3/4) for **Quattro** from the Status Screen to view the Profiles Screen. Select the test profile for "Malaria PvPf" to be run from the Profiles Screen on the Analyzer screen.
4. Enter the patient details as prompted in the **Truelab**® Analyzer screen.
5. Press Start Reaction.
6. For **Truelab**® **Uno Dx,** Press the eject button to open the chip tray. For **Truelab**® **Duo/Quattro**, the chip tray opens automatically on tapping the "Start Reaction" button.
7. Open a pouch of **Truenat**® **Malaria Pv/Pf** and retrieve the micro PCR chip, microtube and DNase & RNase free pipette tip.
8. Place the **Truenat**® **Malaria Pv/Pf** chip on the chip tray without touching the white reaction well. The reaction well should be facing up and away from the Analyzer. Gently press the chip to ensure that it has seated in the chip tray properly.
9. Place the microtube containing freeze dried PCR reagents in the microtube stand provided along with the **Truelab**® Real Time micro PCR workstation **after ensuring that white pellet of dried PCR reagents remains at the bottom of the microtube.** Remove the microtube cap and dispose it off as per the section on "Disposal and Destruction" (Section 17). Using the filter barrier tip provided in the pouch, pipette out six (6) µL of the purified DNA from the Elute Collection Tube into the microtube. Allow it to stand for 30-60 seconds to get a clear solution. ⚠ Do not mix it by tapping, shaking or by reverse pipetting. Using the same filter barrier tip, pipette out six (6) µL of this clear solution and dispense into the centre of the white reaction well of the **Truenat**® **Malaria Pv/Pf** chip. Take care not to scratch the internal well surface and not to spill elute on the outside of the well. Dispose off the microtip as per the section on "Disposal and Destruction" (Section 17).
10. For **Truelab**® **Uno Dx,** slide the chip tray containing the **Truenat**® **Malaria Pv/Pf** chip-based Real Time PCR test loaded with the sample into the **Truelab**® Analyzer. Press Done on the "Please Load Sample" Alert message. For **Truelab**® **Duo/Quattro**, select "YES" at the Please load Sample prompt. Chip tray will close automatically and the reaction will start.
11. Read the result from the screen.
12. After the reaction is completed, for **Truelab**® **Uno Dx,** push the Eject button to eject the chip tray. For **Truelab**® **Duo/Quattro**, tap the "Open/Close Tray" button to eject the chip tray.
13. Take out the **Truenat**® **Malaria Pv/Pf** micro PCR chip at end of the test and dispose it off as per the section on "Disposal and Destruction" (Section 17).
14. Turn on **Truelab**® micro PCR printer and select print on the screen for printing out hard copy of the results. Test results are automatically stored and can be retrieved any time later. (Refer to **Truelab**® Analyzer manual).
15. Switch off the **Truelab**® Analyzer.

15. RESULTS & INTERPRETATIONS

Three amplification curves are displayed on the **Truelab**® Real Time micro PCR Analyzer screen to indicate the progress of the test. Either or both the target and the internal positive control (IPC)* curves will take a steep, exponential path when the fluorescence crosses the threshold value in case of positive samples. The Ct will depend on the number of parasite genomes in the sample. The target curve will remain horizontal throughout the test duration and the IPC curve will take an exponential path in case of negative samples. In case the IPC curve remains horizontal in a negative sample, the test is considered as Invalid. At the end of the test run, the results screen will display "DETECTED" for Positive result or "NOT DETECTED" for Negative result. The result screen would also display the Ct value and the parasite load in copies/mL for positive specimen. The result screen also displays the validity of the test run as "VALID" or "INVALID". Invalid samples have to be repeated with fresh specimen from the sample preparation stage. *While IPC will co-amplify in most positive cases also, in some specimen having a high target load, the IPC may not amplify, however the test run is still considered valid.

16. QUALITY CONTROL PROCEDURES

To ensure that the **Truelab**® Real Time micro PCR Analyzer is working accurately, run

positive and negative controls from time to time. The **Truenat®** Positive Control kit-Panel IV (REF 801040008) containing Positive Control and Negative Control must be ordered separately. It is advisable to run controls under the following circumstances: • Whenever a new shipment of test kits is received. • When opening a new test kit lot. If the temperature of the storage area falls outside of 2-30°C. • By each new user prior to performing testing on clinical specimen.

17. DISPOSAL AND DESTRUCTION

1. Submerge the used content such as **Truenat® Malaria Pv/Pf** chip, microtube, microtube cap, transfer pipette, pipette tips, lysis buffer tube etc. in freshly prepared 0.5% sodium hypochlorite solution for 30 minutes before disposal as per the standard medical waste disposal guidelines.
2. Disinfect the solutions and/or solid waste containing biological samples before discarding them according to local regulations.
3. Samples and reagents of human and animal origin as well as contaminated materials, disposables, neutralized acids and other waste materials must be discarded according to local regulations after decontamination by immersion in a freshly prepared 0.5% of sodium hypochlorite for 30 minutes (1 volume of 5% sodium hypochlorite for 10 volumes of water).
4. Do not autoclave materials or solutions containing sodium hypochlorite.
5. Chemicals should be handled in accordance with Good Laboratory Practice and disposed off according to the local regulations.

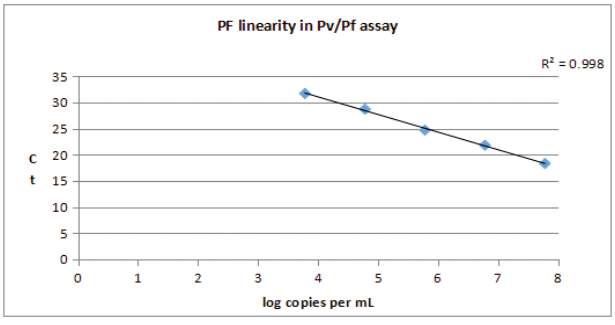
18. SPECIFIC PERFORMANCE CHARACTERISTICS

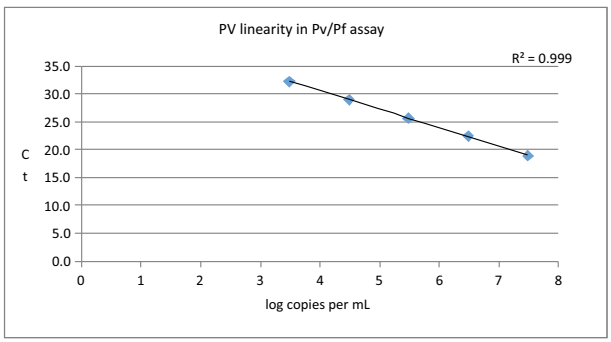
Analytical Exclusitivity (Primer specificity): The following microorganisms were evaluated *in silico* from the NCBI database using the NCBI nucleotide blast and primer blast tools to determine potential cross-reactivity in the **Truenat® Malaria Pv/Pf** assay. Results obtained showed no cross reactivity of the **Truenat® Malaria Pv/Pf** assay with the listed organisms.

Bacteria	Virus
Acinetobacter anitratus	Human Papilloma virus
Candida albicans	Cytomegalovirus
Gardnerella vaginalis	Hepatitis B virus
Enterobacter cloacae	Epstein-Barr virus
Salmonella enterica	Simian virus
Staphylococcus aureus	Human Immunodeficiency virus
Neisseria gonorrheae	Hepatitis C virus
Escherichia coli	Herpes Simplex virus
Streptococcus mutans	Adenovirus
Enterococcus faecalis	
Trichomonas vaginalis	

Linearity:

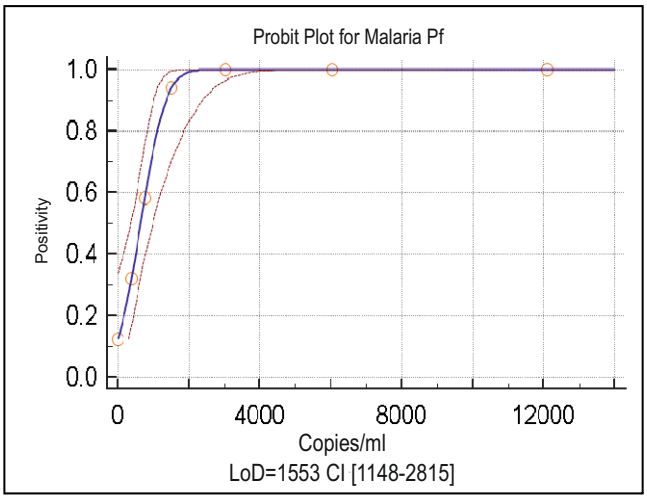
Serial dilutions of *Plasmodium falciparum* plasmid DNA (from 6.1E+07copies/mL to 6.1E +03copies/mL)and *Plasmodium vivax* plasmid DNA (from 3.1E+07copies/mL to 3.1E +03copies/mL) were made and nucleic acids were extracted on **Trueprep® AUTO** Universal Cartridge Based Sample Prep Device in triplicates for each dilution followed by PCR on **Truelab® Uno Dx** Real Time micro PCR analyzer. The assay is found to be linear over 5 orders of magnitude (from 6.1E+07 copies/mL to 6.1E +03 copies/mL) for *Plasmodium falciparum* DNA.Similarly, the assay is found to be linear over 5 orders of magnitude (from 3.1E+07 copies/mL to 3.1E +03 copies/mL) for *Plasmodium vivax* DNA by **Truenat® Malaria Pv/Pf** test as depicted in the given graphs.

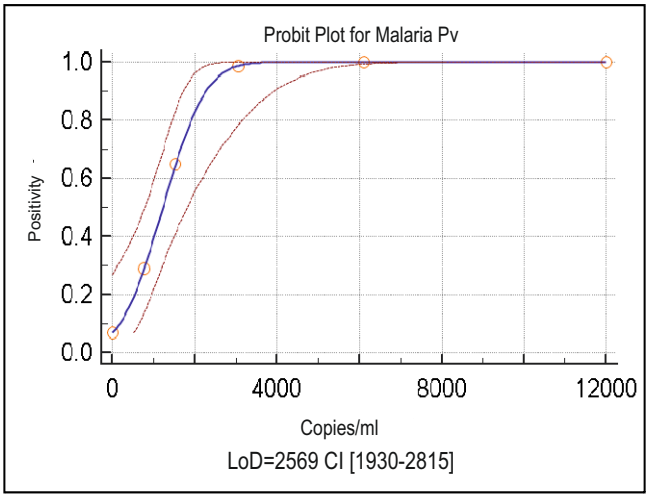




Limit of detection (LoD):

The LoD was determined by testing dilutions of *Plasmodium falciparum* and *Plasmodium vivax* plasmid DNA. Probit analysis of the data was used to determine the concentration of the DNA with 95% probability of detection. The LoD was found to be 1553 copies/mL for *Plasmodium falciparum* plasmid DNA and 2569 copies/mL for *Plasmodium vivax* plasmid DNA.





Robustness:

To determine whether the **Truenat® Malaria Pv/Pf** chip-based Real Time PCR test showed any signs of carryover of PCR products between runs, alternating runs of positive samples and negatives samples were performed. 10 positive samples and 10 negative samples were used for the study. The **Truenat® Malaria Pv/Pf** test did not exhibit detectable carryover contamination between positive to negative sample runs.

Reproducibility:

The purpose of this study is to compare the functional performance of the **Truenat® Malaria Pv/Pf** assay using three different titres of samples on **Truelab® Uno Dx** Real Time micro PCR analyzer. High, Medium and low titre samples were extracted on **Trueprep® AUTO** Universal Cartridge Based Sample Prep Device and tested among three different users (Inter user), on three different devices (Inter device) and on 5 consecutive days (Inter day) to check the variability. Mean %CV values for all titres has been calculated for Malaria Pf as Inter User (2.04), Inter day (1.95) and Inter Device (1.86) and for Malaria Pv as Inter User (3.93), Inter day (2.04) and Inter Device (2.67) which were in the accepted range of ≤15% CV for **Truenat® Malaria Pv/Pf** assay.

Precision of Truenat® Malaria Pv/Pf assay:

Precision was tested by performing **Truenat® Malaria Pv/Pf** assay with extracted DNA of High (6.1E+05 copies/mL), Medium (6.1E+04 copies/mL) and Low (6.1E+03 copies/mL) titres for Malaria Pf while High (3.1E+06 copies/mL), Medium (3.1E+05 copies/mL) and Low (3.1E+04 copies/mL) titres for Malaria Pv for five consecutive days. Every day PCR for each titre DNA was run in triplicates. The %CV values obtained for High titre (1.69), Medium titre (1.45) and low titre (2.94) for Malaria Pf while High titre (2.66), Medium titre (3.32) and low titre (3.56) for Malaria Pv were within the accepted range of ≤15% CV for **Truenat® Malaria Pv/Pf** assay.

19. REFERENCES

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SYMBOL KEYS

Consult instructions for use	IVD *In vitro* Diagnostic Medical Device. Not for medicinal use.	Temperature Limitation	REF Catalogue Number	For single use only	This Side Up	Manufacturer
Date of Manufacture	Date of Expiry	LOT Batch Number / Lot Number	Caution	Σ Contains sufficient for <n> tests	EC REP Authorised Representative in the European Community	

Molbio Diagnostics Private Limited

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