APPENDICES

Appendix 1

Consent form (Thai)

ใบยินยอม

คณะผู้วิจัย: SMRU (Shoklo Malaria Research Unit)

วัตถุประสงค์: เพื่อประเมิณผลการศึกษาชุดตรวจวินิจฉัยมาลาเรียแบบใหม่

ข้าพเจ้าและบุตรของข้าพเจ้าเข้าใจและยินยอมเข้าร่วมเป็นอาสาสมัครในการวิจัยนี้ทั้งนี้ข้าพเจ้าหรือบุตรของข้าพเจ้าป่วยเป็นไข้มาลาเรีย

ข้าพเจ้ายินยอมให้คณะผู้วิจัยของ SMRU เก็บตัวอย่างเลือดจากปลายนิ้วของข้าพเจ้าประมาณ 250 ไมโครลิตรหรือประมาณ 2-3 หยดเพื่อใช้ในการวิจัยเรื่องการตรวจวินิจฉัยไข้มาลาเรีย

ในกรณีที่ได้มีการวินิจฉัยไข้มาลาเรียแล้วข้าพเจ้ายอมให้มีการเจาะเลือดปริมาณไม่เกิน 6 มิลลิลิตรเพื่อนำไปใช้ในการวิจัยขั้นต่อไปข้าพเจ้าเข้าใจว่าข้าพเจ้าจะได้รับแจ้งการเปลี่ยนแปลงต่างๆอันเกี่ยวเนื่องกับงานวิจัยดังกล่าวข้างต้น ในกรณีที่มีการเปลี่ยนแปลงหากข้าพเจ้ามีข้อสงสัยประการใดผู้เชี่ยวชาญจาก SMRU จะเป็นผู้ตอบคำถามต่างๆแก่ข้าพเจ้า

การศึกษานี้มีจุดมุ่งหมายเพื่อนำมาซึ่งข้อมูลใหม่ๆเกี่ยวกับการตรวจวินิจฉัยไข้มาลาเรียแบแใหม่และไม่มีผลประโยชน์โดยตรงต่อข้าพเจ้า

ข้าพเจ้ายินยอมให้นำผลการวิจัยนี้ไปตีพิมพ์เผยแพร่เพื่อวัตถุประสงค์ทางวิชาการโดยมิให้มีการเปิดเผยชื่อของข้าพเจ้าในสิ่งตีพิมพ์นั้น

ข้าพเจ้าเข้าใจดีว่าคณะกรรมการด้านจริยธรรมในงานวิจัยทางวิทยาศาสตร์ได้ทำการกลั่นกรองและเห็นชอบในงานวิจัยนี้ข้าพเจ้าเข้าใจดีว่าข้าพเจ้ามีสิทธิในการถอนตัวและสิ้นสุดฐานะการเป็นอาสาสมัครในการวิจัยนี้ได้โดยตลอดเวลา

ข้าพเจ้าทราบดีว่าข้าพเจ้าจะไม่ได้รับผลตอบแทนใดๆในการร่วมเป็นส่วนหนึ่งของงานวิจัยนี้

_____ ____/____/_____

ลายเซ็นชื่อผู้ยินยอม วันที่

_____

ชื่อผู้ยินยอม (ตัวพิมพ์)

Appendix 2

Consent form (English)

Consent Form

The Investigators are:	**SMRU** (Shoklo Malaria Research Unit)
Purpose:	The purpose of the study is to evaluate a new diagnostic test for MALARIA

Permission

I understand that I was selected, or my child was selected as a volunteer for this study because he, she or I have MALARIA.

I hereby authorize a qualified person designated by the SMRU to withdraw 250 µL (a few drops) from a finger prick for the purpose of medical research related to the diagnosis of MALARIA.

In case the diagnostic of MALARIA is established, no more than 6 mL will be removed. I understand that I will be informed of any change in the nature of the study or the procedures, as described above, as they may occur. A qualified person from SMRU will answer any questions that I have.

I understand the purpose of these tests is to gain information about the potential of a new diagnostic test for malaria, and they are not intended as a direct benefit to me.

I consent to the use of the results of the studies performed with this sample of blood for publication for scientific purposes, excluding my identity.

I understand that a committee exists which has reviewed, and continues to review this study from a scientific and ethical standpoint. I further understand that I am free to withdraw my voluntary consent and discontinue my volunteer participation at any time without prejudice.

I understand that there is no compensation available for my participation in this research study.

_____ ___/___/____

DONOR'S Signature Date:

_____

Donor name (printed)

Appendix 3

Consent form (Karen)

ပုၤဆါတၢ်ဘဉ်သးလံာ်ဒိ

ပုၤယိထံသ့ဉ်ညါတၢ် SMRU

တၢ်ပညီဉ် တၢ်ယိထံမၤလိတဘျိအံၤဒ်သိးကထိဉ်တယာ်တၢ်အဝိးအလိလၢတၢ်ယုဟ်ဖျါညဉ်ဂိၢ်တၢ်ဆါအဂီၢ်.

<u>တၢ်ဟ့ဉ်လီၤန့ဲး</u>

ယနၢ်ပၢၢ်လၢ ယၤမ့တမ့ၢ်ယဖိအံၤဘဉ်တၢ်ယုထၢအီၤဒ်သိးကနာ်လီၤဆူတၢ်ယိထံမၤလိတဘျိအံၤလၢပကစၢ်တၢ်ဘဉ်သးဒဉ်ပဝ်လီၤ. ဘဉ်သ့ဉ်သ့ဉ်ပကအိဉ်ဒီးတၢ်ညဉ်ဂိၢ်အယၢ်အယိပဘဉ်တၢ်ယုထၢအီၤလီၤ.

လၢတၢ်နှဉ်အယိယဟ့ဉ်စိဟ့ဉ်ကမီၤပုၤမၤတၢ်ဖဲ(SMRU)ဒ်သိးကဆဲးဖျိယစုနၢစိးဒီးဟံးန့ၢ်သွံဉ် 250 µL ဒ်သိးကယုမၤကွၢ်ဘဉ်ဃဉ်ဒီးတၢ်ယုဟ်ဖျါတၢ်ညဉ်ဂိၢ်တၢ်ဆါလီၤ.

ပုၤမ့ၢ်ထံဉ်နှ့ၢ်တၢ်ညဉ်ဂိၢ်အယၢ်တခီပုၤကထုးန့ၢ်သွံဉ် 6 ml လီၤ. တၢ်ဆီတလဲမ့အိဉ်ထိဉ်တမံၤလဉ်လဉ်လၢတၢ်မၤကွၢ်အကျိၤအကျဲနှဉ်ဒီးယကဘဉ်တၢ်ဒုးသ့ဉ်ညါအီၤလီၤ. ယမ့ၢ်အိဉ်ဒီးတၢ်သံကွၢ်တမံၤလဉ်လဉ်ဒီးပုၤဘဉ်မူဘဉ်ဒါလၢ SMRUန့ဉ်ကဟ့ဉ်ယၤတၢ်စံးဆၢလီၤ.

ယနၢ်ပၢၢ်လၢခီဖျိတၢ်ဟံးန့ၢ်သွံဉ်ခံတၢ်ဘဉ်ဟ်ဖျါအီၤလၢထးအသိးန့ဉ်တၢ်လၢလာ်သ့ဉ်တဖဉ်ကဲထိဉ်သ့လီၤ. တၢ်ပနၤထိဉ်၊ တၢ်အဃၢ်နှာ်လီၤ၊ တၢ်မဲာ်ခံးသူ၊ အဆါ ဒီး တၢ်တမ့ာ်တလၤတဖဉ်. တသ့ဖဲအသ့ဒ်သိးတၢ်သ့ဉ်တဖဉ်အသုတကဲထိဉ်တဂ့ၤအဂီၢ်ပုၤကအိဉ်ဒီးတၢ်ပလီၢ်ပဒိစ့ၢ်ကီးနှဉ်လီၤ. ယနၢ်ပၢၢ်လၢတၢ်မၤကွၢ်တဘျိအံၤအတၢ်ပညီဉ်နှဉ်ဒ်သိးကသ့ဉ်ညါအါထိဉ်ဘဉ်ဃးတၢ်ယုဟ်ဖျါတၢ်ညဉ်ဂိၢ်အယၢ်အဝိးအလိအသိအံၤအဂ့ၢ်လီၤ. တၢ်အံၤတၢ်ဘဉ်ဘျူးလၢယဂိၢ်လိၤလိၤဒုတအိဉ်ဘဉ်.

ယဟ့ဉ်အခွဲးလၢတၢ်မၤကွၢ်အစၢနှဉ်ပုၤရၤလီၤလၢလံာ်လဲဉ်အပူၤဒ်သိးကကဲဘျူးလၢစဲအ့ဉ်တၢ်ကူဉ်ဘဉ်ကူဉ်သ့အဂီၢ်ဘဉ်ဆဉ်တဘဉ်သူယမံၤယသဉ်ဘဉ်.

ယနၢ်ပၢၢ်လၢတၢ်ယိထံမၤကွၢ်တဘျိအံၤအိဉ်ဒီးအကမံတံာ်လၢကွၢ်ကဒါကှၤတၢ်ဖံးတၢ်မၤဒီးကကွၢ်ကဒီးဝဲဆူညါလၢစဲအ့ဉ်ဒီးပုၤကညီဂ့ၢ်ဝီအသနၢဉ်နှဉ်လီၤ. တဘျိလဉ်လဉ်ဖဲယအဲဉ်ဒိးဟးထိဉ်လၢတၢ်မၤကွၢ်အံၤဃဟးထိဉ်ဃဲသ့ဒီးတၢ်လၢကမၤတြိတံာ်ကးယၤတအိဉ်လၢကျဲနီတဘိဘဉ်.

ယနၢ်ပၢၢ်လၢခီဖျိယတၢ်နာ်လီၤမၤသကိးတၢ်တဘျိအံၤအဘူးအလဲလၢယကဒိးနှ့ၢ်အီၤတအိဉ်ဘဉ်.

_____ ____/____/_____

ပုၤဟ့ဉ်မၤဘူဉ်တၢ်ဆဲးလီၤမံၤ မ့ၢ်နံၤမ့ၢ်သီ

_____

ပုၤဟ့ဉ်မၤဘူဉ်တၢ်အမံၤ

Appendix 4

Microscopy form I

OptiMAL IT Study

Microscopy Form I (MKT)

Code OPT _____ ☐ OPD _____

☐ TMI _____

☐ HP _____

Date_____

Name_____ Sex _____M_____F

Age _____years

Microscopy results

Hematocrit _____%

Number of asexual parasites/µL of blood _____ /µL

Sample taken

- ☐ PCR spots
- ☐ EDTA blood 1 ml

Lab tech name_____

Appendix 5

Microscopy form II

OptiMAL IT Study

Microscopy Form I I (MS)

Code OPT _____ ☐ OPD _____

☐ TMI _____

☐ HP _____

Date_____

Name_____

Microscopy results (2nd level)

Lab tech name_____

Appendix 6

Microscopy form III

OptiMAL IT Study

Microscopy Form I I I (3rd Level)

Code OPT _____ ☐ OPD _____

☐ TMI _____

☐ HP _____

Date_____

Name_____

Request for the 3rd level

Microscopy results

Lab tech name_____

Appendix 7

OptiMAL Result form

OptiMAL IT Study

OptiMAL Result Form

Code OPT _____ ☐ OPD _____

☐ TMI _____

☐ HP _____

Date_____

Name_____

OptiMAL Result

_____ Negative

_____ Positive _____ P.falciparum, Intensity

☐ Slightly positive ☐ Positive ☐ Strongly positive

_____ Positive _____ P. non-falciparum, Intensity

☐ Slightly positive ☐ Positive ☐ Strongly positive

_____ Invalid

Remark

Lab tech name _____

Appendix 8

Paracheck result form

OptiMAL IT Study

Paracheck-Pf Form

Code OPT _____ ☐ OPD _____

☐ TMI _____

☐ HP _____

Date_____

Name_____

Paracheck -Pf

_____ Negative

_____ Positive ☐ Slightly positive

☐ Positive

☐ Strongly positive

_____ Invalid

Remark

Lab tech name _____

Appendix 9

EDTA List

OptiMAL IT Study

EDTA List

NO.	CODE	Date of collection
1	OPT	
2	OPT	
3	OPT	
4	OPT	
5	OPT	
6	OPT	
7	OPT	
8	OPT	
9	OPT	
10	OPT	
11	OPT	
12	OPT	
13	OPT	
14	OPT	
15	OPT	
16	OPT	
17	OPT	
18	OPT	
19	OPT	
20	OPT	
	Total	samples

Appendix 10

PCR List

OptiMAL IT Study

PCR List

NO.	CODE	Date of collection
1	OPT	
2	OPT	
3	OPT	
4	OPT	
5	OPT	
6	OPT	
7	OPT	
8	OPT	
9	OPT	
10	OPT	
11	OPT	
12	OPT	
13	OPT	
14	OPT	
15	OPT	
16	OPT	
17	OPT	
18	OPT	
19	OPT	
20	OPT	
	Total	samples

Appendix 11

OptiMAL Time Record

OptiMAL-IT Time Record **Date _____**

1. OPT _____	**11. OPT_____**
2. OPT _____	**12. OPT _____**
3. OPT _____	**13. OPT_____**
4. OPT _____	**14. OPT_____**
5. OPT _____	**15. OPT_____**
6. OPT _____	**16. OPT_____**
7. OPT _____	**17. OPT_____**
8. OPT _____	**18. OPT_____**
9. OPT _____	**19. OPT_____**
10.OPT _____	**20. OPT_____**

Appendix 12

Microscopic Examination Procedure

Blood sample will be collected by finger prick from clinically suspected malaria cases presenting at the OPD.

Thick and thin blood film will be prepared on the same slide and stained with fresh 10% Giemsa solution at pH 7.2 for 20 minutes. (Two slides will be taken from each patient; one slide will be examined at the first level (on site) and second level (Mae Sod) the other will be kept for the third level at HTD for further examination).

Thick blood film will be examined by using 1000 X magnification by expert microscopist as the standard practice. Parasitaemia will be measured by counting the number of asexual parasites against a number of 500 leukocytes in the thick blood film by hand two-tally counter; if parasite count > 500/500 WBC the count will be done against 1000 RBC in the area where RBC are normally distributed in thin smear. Parasites stage and species identification will be done on the thin films.

Thick films with no parasite found in 200 fields, will be considered to be negative.

Assuming that the average WBC count is 8000 per microliter of blood, the parasitaemia will be calculated by using the formula:

$$\text{Parasitaemia}/\mu L = \frac{\text{Number of parasites per 500 WBC} * 8000}{500}$$

For parasitaemia done on thin smear (against RBC), the following formula will be used:

Parasitaemia/μL = Number of infected RBC per 1000 RBC * Hct * 125.6

e.g. 15 per 1000 RBC with Hct = 36 %

Parasitaemia/ μL = 15 * 36 * 125.6

= 67,824 parasites/μL

Slides will be reexamined in the same way by a second microscopist at the secondary level (Mae Sod) without knowing the result of the first level.

Appendix 13

OptiMAL-IT® Assay Procedure

The OptiMAL-IT test were kept in room temperature and just opened before performing to avoid exposing to humidity. The patient's code and date was labeled on the device. The same finger-prick blood wound from which malaria smear were made was used for the blood collection by the pipette supplied with the kit. The entire volume of blood (approx. 10 μL) was added to the Conjugate well. After mixed gently, the test was stood for 1 minute. The test device was pulled out and the dipstick vertical was inserted into the conjugate well and stood for 10 minutes or until the blood or conjugated mixture completely soaked up. After the blood migrated toward the filter pad, transferred the dipstick to the wash well and waited for 10 minutes until the filter pad is completely cleared of blood. The time had to be 10 minutes if less than that there could be a back flow of blood though the pad is completely cleared. Then removed the dipstick from the wash well and clicked it back into the device. Cleared strip was read under natural daylight and the result was recorded in the form and on the device.

Appendix 14

Paracheck Pf® Assay Procedure

Paracheck test were kept in room temperature and just opened before testing. The patient's code, date and exact time of testing were recorded on the device. From the same finger-prick, the adequate quantity of blood (approx. 5 μL) was collected by pipette provided in the kit. Applied the blood to the sample well "A" immediately then dispensed 6 drop of clearing buffer into well "B". The result was read at the end of 15 minutes under natural daylight and was recorded in the form and on the device.

Appendix 15

OptiMAL IT® Leaflet

87100000 01.08

DiaMed

English

Temporary - Boxe Insert (0201b)
Only for evaluation use

OptiMAL-IT

More than 500 million cases of Malaria occur each year, of which 3 million prove fatal, particularly in children.

DiaMed **OptiMAL-IT** is a dipstick test for the detection of an infection by Plasmodium spp in human blood samples with differentiation between: ***P. falciparum*** (responsible agent of most fatal cases) and ***P. non falciparum*** (*P. vivax, P. ovale* or *P. malariae*).

Specificity: OptiMAL-IT detects the presence of pLDH (parasite lactate dehydrogenase), an enzyme produced by both sexual and asexual forms of the parasite. There is no cross-reaction with the human LDH.

Sensitivity: OptiMAL-IT detects peripheral parasitaemia levels of 0.001-0.002% (50-100 parasites per µL of blood). The sensitivity can be compared with microscopic observation of a thin blood smear, using a x100 immersion objective, for a period of approx. 30 minutes, by a well-trained technician.

Fast and Easy : The result is obtained in 20 minutes. **OptiMAL-IT** provides all necessary materials to perform a test, ideal for a non-laboratory environment in endemic areas.

Monitoring of treatment with OptiMAL-IT: the test is only positive when live parasites are present in the blood. A repeat test becomes negative generally within 2-4 days following the beginning of successful treatment. Thus **OptiMAL-IT** is suitable to verify the effectiveness of therapy, underlining possible resistant strains of Plasmodium spp.

Reagents

OptiMAL-IT : individual rapid Malaria test

each single test package contains:

1 device with dipstick, conjugate well, wash well
1 well cover
1 dropper ampoule of buffer
1 lancet
1 disinfectant swab
1 pipette (calibrated for 10 µL)
1 schematic test procedure

In boxes of 12 individual Test packages

Stability: see expiry date on aluminium foil.

Important: When transporting or storing the packages, avoid exposure to high temperature (over 38°C) for a period longer than 1 day. Avoid any exposure to 60°C and higher (the reagents may be damaged).

Caution: When used as directed, **OptiMAL-IT** reagents present no risk to the user; after contact with patient's blood sample discard the potential infected material into suitable waste container. Do not pipette by mouth.

Sample Material

- Capillary blood collected from fingertip or from ear. See test procedure below.
- Fresh whole blood, collected by venipuncture using EDTA or Heparin sample tubes.

Positive Control

DiaMed **OptiMAL** Positive control, prepared from in vitro cultures of Plasmodium falciparum, contains pLDH specific for Plasmodium falciparum (separately available).

It is recommended to verify the performance of **OptiMAL-IT** in a regular manner, and especially after transportation and storage under tropical conditions.

Principle of the test

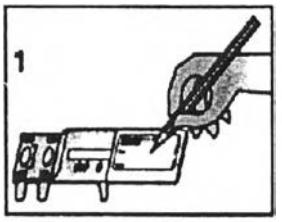
DiaMed-**OptiMAL-IT** is a rapid detection test using a dipstick coated with monoclonal antibodies against the metabolic enzyme parasite lactate dehydrogenase (pLDH).
In case of presence of Plasmodium in the blood sample, the pLDH captured by the conjugate reacts with the specific antibodies against P.falciparum and/or Plasmodium spp. The reactions are demonstrated by the appearance of dark purple bands on the dipstick. The band of the procedure control (goat anti-mouse) must always be present to validate a result. In case of absence of the control band, the test is not valid.

1) Goat anti-mouse (procedure control)

2) Monoclonal anti-pLDH specific to all 4 species of Plasmodium (*P. falciparum, vivax, malariae, ovale*)

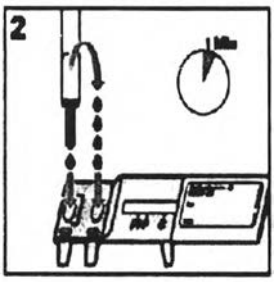
3) Monoclonal anti-pLDH specific to *P. falciparum*

Test procedure



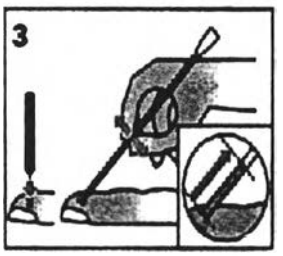
Tear open the aluminium package and take out all the material.
Important: Don not leave the material exposed to humidity and high temperature over 1 hour. Avoid exposure to sunlight.

1. Take the device, place it horizontally on a flat surface, write the **patient's name** or number on the label.



2. Tear open the buffer-ampoule and add:
   - **1 drop of buffer** (approximately 20 µL) in the first well (Conjugate, marked with a red line)
   - **4 drops of buffer** (approximately 80 µL) to the second well (Wash well)

   Allow to stand for 1 minute.



3. **Blood collection:** collect blood sample from finger prick. Use the Lancet and the Pipette supplied with the kit or use fresh blood from tubes containing anti-coagulant (EDTA, heparin).

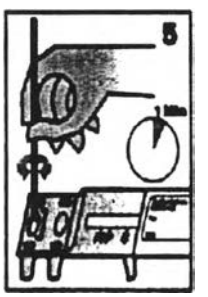
   Finger prick: Aseptically clean the fingertip with the disinfectant swab, let dry, remove the lancet from its envelope, prick the lateral part of the fingertip.

   Take the Pipette, squeeze it, place the open tip into the blood drop, release pressure and draw up blood to the black line.
   Discard the used swab and the Lancet into a suitable waste container.

   When using **venous blood**, draw blood from the tube into the Pipette in the same manner.

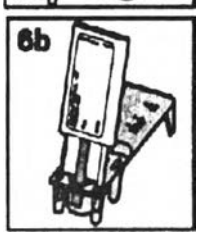
4. Add the entire volume of blood (approx. 10 µL) by squeezing the Pipette gently, to the 1 first well (Conjugate Well with the mark around).

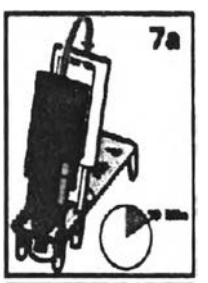
5. Mix gently with the Pipette (upper end) and allow to stand **for 1 minute.**
   Discard the Pipette into a suitable waste container.



6a. Open the IT-device: hold the device with the wells between thumb and forefinger and, with the other hand, pull out the flat piece (with the label). Place the wells back on the table, and insert the dipstick vertical into the Conjugate Well (with red line).
Allow to stand for **10 minutes**, so that the blood/conjugate mixture is completly soaked up.

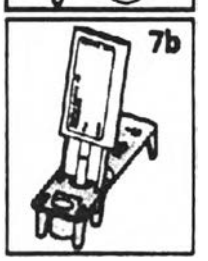
6b. The blood migrates towards the filter pad and the control band will appear progressively.

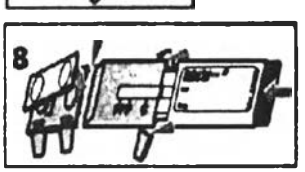




7a. Transfer the Dipstick to the second well (Wash well) and allow to stand for **5-10 minutes**, until the reaction field is completely cleared of blood.

7b. The control band (marked C) must now be clearly visible.





8. Remove the dipstick from the wash well and click it back into the clear plastic piece.
   Close the wells with the well cover, break it off, and break the two legs off from the clear plastic piece.
   Discard them into a suitable waste container.

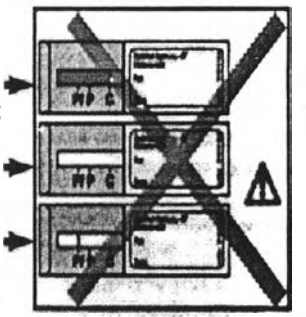
9. Read the reaction:
   first validate the test and then interprete the results (see: Interpretation of the results).
   The Dipstick slide should be kept for future reference and for comparison in case of further tests for monitoring the efficiency of treatment.

Interpretation of the results

1) Validation of the obtained results

The test is not valid in three cases:

- when the Dipstick is not sufficiently cleared (reaction field remains red), the volume of buffer may have been insufficient or the washing time too short
- when the control band does not appear
- when the control band does not appear even if one, or both of the diagnostic bands becomes positive.



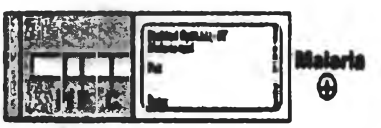
Repeat the test, following precisely the test procedure!

2) Interpretation of the reaction pattern

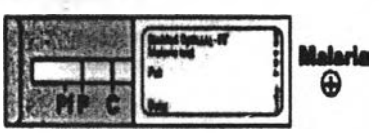
Negative reaction:
no pLDH is detected in the sample.
No reaction occurs with the pLDH antibodies and only the control band will be visible.

Positive reactions:
the pLDH present in the sample reacts with the anti-pLDH conjugate and rises up the Dipstick where it is captured by one or both of the specific pLDH antibodies, causing the appearance of a coloured band.

- **Positive for *Plasmodium falciparum***
and the possibility of a mixed infection with: *P. vivax*, *P. ovale* or *P. malariae*, can not be excluded.



- **Positive for non *Plasmodium falciparum* species:**
P. vivax or *P. ovale* or *P. malariae*.
This sample is negative for *P. falciparum*.



NB: Monitoring of treatment

Repeat the test 2 days (48 hours) after start of the treatment, and again 2-3 days later, repeat testing and compare the reactions with the results obtained before treatment.

If the reaction remains positive after 7-10 days, the possibility of a resistant strain should be considered.

Limitations

OptiMAL-IT detects the presence of pLDH from living parasites only (hence the possibility to monitor the efficiency of treatment). Comparison tests with PCR may therefore not be conclusive. Any modifications to the described test procedure or use of other reagents may modify the reaction pattern and invalidate the test.

Remark

The unused dipsticks present faint lines at the position of the 3 antibodies. These faint lines are present for in process control and quality control during the manufacturing process. They disappear completely as soon as the reaction field of the dipstick is in contact with the blood sample and conjugate.

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Products			
	OptiMAL-IT	1 x 12 individual tests	cat. no. 710021
	OptiMAL Positive control	8 wells	cat. no. 710011
	OptiMAL-Teaching kit	1 piece	cat. no. 710022

Manufactured by: DiaMed SA, 1786, Cressier sur Morat, Switzerland. *under license from FLOW Inc., Portland, OR 97201, USA.*
www.diamed.ch/optimal

EC authorised representative: DiaMed France S.A., 9 Rue Française, F-75002 Paris.

Appendix 16

Paracheck PF® Leaflet

paracheck Pf

RAPID TEST FOR *P. falciparum* MALARIA

(Device)

INTRODUCTION

Paracheck Pf is a rapid self performing, qualitative, two site sandwich immunoassay for the determination of *P. falciparum* specific histidine rich protein - 2 (Pf HRP-2) in whole blood samples.

SUMMARY

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

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

PRINCIPLE

Paracheck Pf is a rapid test for the detection of *P. falciparum* malaria that utilizes the principle of immunochromatography. As the test sample flows through the membrane assembly of the device after addition of the clearing buffer, the colored anti Pf HRP-2 colloidal gold conjugate (monoclonal) antisera complexes the Pf HRP-2 in the lysed sample. This complex moves further on the membrane to the test region where it is immobilised by the anti Pf HRP-2 (monoclonal) antisera coated on the membrane leading to formation of a pink colored band which confirms a positive test result. Absence of this colored band 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 immobilised by antimouse antibodies coated on the membrane at the control region, forming a pink band. This control band serves to validate the test performance.

REAGENTS AND MATERIALS SUPPLIED

Paracheck Pf kit contains :

1. Individually pouched devices : Membrane assembly predispensed with anti Pf HRP-2 colloidal gold conjugated antisera, anti Pf HRP-2 antisera and anti mouse antisera at the respective regions and sample applicator pipette.
2. Clearing buffer in a dropper bottle.

OPTIONAL MATERIAL REQUIRED

Calibrated micro pipette capable of delivering 5 μl sample accurately.

STORAGE AND STABILITY

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

NOTE

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

SPECIMEN COLLECTION AND PREPARATION

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

TEST PROCEDURE

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

   OR

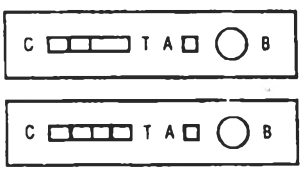
   In case finger prick blood is being used, touch the sample applicator pipette to the blood on the finger prick and immediately blot the specimen on to the sample pad in the sample well 'A' (Care should be taken that the blood sample has not clotted and the transfer to the sample pad is immediate).

OR

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

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

5. Dispense six drops (300μl) of the clearing buffer into well 'B' by holding the plastic dropper bottle vertically.
6. At the end of 15 minutes read the results as follows:



NEGATIVE for *P. falciparum* malaria : Only one pink colored band appears in the control window 'C'.

POSITIVE for *P. falciparum* malaria : In addition to the control band, a distinct pink colored band also appears in the test window 'T'.

7. The test results should not be interpreted after 15 minutes.
8. The test should be considered invalid if no bands appear on the device. Repeat the test with a new device ensuring that the test procedure has been followed accurately.

LIMITATION OF THE TEST

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

PERFORMANCE CHARACTERISTICS

1. In an independent study, a panel of 167 samples whose results were earlier confirmed with expert microscopy were tested with **Paracheck Pf** and the results obtained are as follows:

Sample type	Total no. of samples tested	Paracheck Pf		Sensitivity	Specificity
		Positive	Negative	%	%
P. falciparum positive	74	73	1	98.6	-
P. vivax positive	8	0	8	-	100
Malaria negative	85	1	84	-	98.8

2. In an another independent study, 125 patient samples from a *P. falciparum* endemic area were tested with **Paracheck Pf** and microscopy (thick and thin smear). **Paracheck Pf** was found to be 100% sensitive and 100% specific to *P. falciparum* against microscopy. All the 31 samples that tested positive for *P. falciparum* under microscopy showed positive results with **Paracheck Pf**. The four *P. vivax* positive samples and the 90 malaria negative samples tested negative with **Paracheck Pf**.
3. In a third independent study, 100 patient samples were tested with **Paracheck Pf**, with another immunochromatographic test for P. *falciparum* and with microscopy. **Paracheck Pf** showed a 99% correlation with microscopy and a 98% correlation with the other immunochromatographic test.
4. From the above results and the results of in house data, **Paracheck Pf** is a highly sensitive and specific test for the diagnosis of *falciparum* malaria.

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5. Data on file : Orchid Biomedical Systems.

Manufactured by :
Orchid Biomedical Systems
Plot No. 88/89, Phase II C, Verna Industrial Estate, Verna, Goa - 403 722, INDIA.

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