K-Malaria P.f/Pan Combo Test

1. Explanation

The K-Malaria P.f/Pan Combo Test is a rapid, qualitative test for detection of histidine-rich protein II antigen (HRP-2) of *Plasmodium falciparum* and Plasmodium lactate dehydrogenase (pLDH) of all four malarial species, *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*, in whole blood of suspected malarial infective cases. The test kit is intended to detect malarial infection for professional *in vitro* diagnostic use only.

The presence of a positive line at "Pf" or "Pf and Pan" indicates *P. falciparum* infection.

The presence of a positive line at "Pan" indicates *P. vivax*, *P. malariae* and *P. ovale* infection, but not *P. falciparum*.

The positive or negative result detected from the specimens needs to be taken into consideration together with patient history and clinical symptoms related to malarial infection.

2. Provided materials

The K-Malaria P.f/Pan Combo Test contains:

1) Individually foil-pouched test cassettes with a desiccant (25 packs)
2) Assay buffer (1 bottle)
3) Instructions for use

3. Test kit and buffer storage

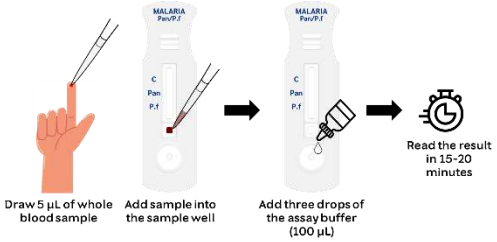
The K-Malaria P.f/Pan Combo Test and buffer should be stored at a temperature of 2-30 °C. The test device is sensitive to humidity and heat. Perform the test immediately after removing the test device from the foil pouch. Do not use the device beyond the expiration date printed on the package.

4. Specimen collection

1. Whole blood collected by fingerstick or by venipuncture can be used with the K-Malaria P.f/Pan Combo Test. Collect whole blood using EDTA anti-coagulant.
2. Whole blood collected by fingerstick should be tested immediately. If whole blood collected by venipuncture using anti-coagulant is not immediately tested, the specimen should be stored at 2-8 °C up to three days, or at -20°C for several months.
3. The specimens should be brought to room temperature prior to use. Frozen samples need to be thawed and mixed well before testing. Do not freeze-thaw samples for more than three cycles.

5. Test procedure

1. Bring the test kit and specimen to appropriate temperature (15-30°C) prior to use.
2. Remove the test cassette from the foil pouch when ready to perform, and place it on clean and flat surface.
3. Draw 5 µL of whole blood sample using pipette or other proper equipment. Add sample into the sample well (Fig.1). Add three drops of the assay buffer (approx. 100 µL), while avoiding bubbling.
4. The red control line should appear in 2 min. If not, add one more drop of buffer.
5. Read the result in 15-20 minutes. Do not read after 30 minutes.
6. If the test result is not legible after 15 minutes due to high background color, read again later, but within 25-30 minutes after adding one more drop of buffer.



7. Caution

1. The test kit is for malaria screening and *in vitro* diagnostic use only.
2. The test kit is intended for professional use or by trained personnel only.
3. Do not eat, drink, or smoke during sample collection.
4. Wear gloves while performing the test. Clean hands before and after performing the test.
5. Clean the used equipment and test area using appropriate disinfectant.
6. Disinfect samples, test device, and contaminated materials after testing. Discard all disinfected materials in a proper biohazard container.
7. Do not use the test device if packaging is broken or pre-opened.
8. Do not use the test kit and buffer after the expiration date.

8. Result and interpretation

Negative:
The presence of only one control line ("C" band) in the result window.

Positive for *P. falciparum*:
The presence of two colored lines: the control "C" band, and the "P.f" band in the result window.

The presence of three colored lines: the control "C" band, the "P.f" band, and the "Pan" band in the result window. This

indicates HRP-2 and pLDH antigens present in the tested specimen.

Positive for non-*P. falciparum* (non-falciparum):
The presence of two colored lines: the control "C" band, and the "Pan" band in the result window.

Invalid test result:
If the color of the control band ("C") is not visible within the result window after performing the test, the result is considered invalid. Repeating the test with a new device is recommended. If the same problem occurs, stop using the test kit, and please contact the distributor.

9. Warning

1. If the result cannot be read within 20 min because of high background color, add one more drop of extracted sample buffer, or read the result again at the 30-min mark. Do not read the result after 30 min. to avoid any misinterpretation.
2. Store the buffer at 2-8 °C after usage.

10. Quality control

1. Internal control: The K-Malaria P.f/Pan Combo Test contains a control band or "C band". The C band develops after adding extracted specimen buffer. If the color of the control band ("C") is not visible after performing the test, the result is considered invalid. Repeating the test with a new device is recommended. If the same problem occurs, stop using the test kit, and please contact the distributor.
2. Positive and negative control: standard positive and negative controls need to be tested to ensure the proper performance of the test, particularly under the following circumstances:
   2.1 A new test kit is used.
   2.2 A new lot of test kits is used.
   2.3 The method of shipment has changed.
   2.4 Storage temperature is outside the range of 2-30°C.
   2.5 Temperature during performance is outside the range of 15-30°C.

11. Performance characteristics

1) Sensitivity and specificity

The K-Malaria P.f/Pan Combo Test was evaluated in 239 clinical whole blood specimens whose malarial identification was confirmed by microscopy examination of thick and thin blood films. The performance of the K-Malaria P.f/Pan Combo Test is shown as follows.

Microscopy examination of thick and thin blood films	K-Malaria P.f/Pan Combo Test		Total
	Positive	Negative	
Positive for *P. falciparum*	69	2	71
Positive for Non-falciparum (*P.vivax*)	67	1	68
Negative	1	124	125
Total	137	127	264

Clinical performance of K-Malaria P.f/Pan Combo Test
Sensitivity to *P. falciparum*: 97.18% (95% CI = 90.19% - 99.66%)
Sensitivity to Non-falciparum: 98.53% (95% CI = 92.08% - 99.96%)
Specificity: 99.20% (95% CI = 95.62% - 99.98%)
Accuracy: 98.48% (95%CI = 96.17 - 99.59%)

2) Precision

1) Intra run: The reproducibility was determined by testing three different replicates of five different specimens containing different concentrations of *P. falciparum* and *P. vivax* antigens with three different lots of K-Malaria P.f/Pan Combo Test simultaneously. The precision was determined to be 100%.

2) Inter run: The reproducibility was determined in different days by testing ten replicates of five different specimens containing different concentrations of *P. falciparum* and *P. vivax* antigens. The precision was determined to be 100%.

3) Chemical interference

Interference substances were used to evaluate K-Malaria P.f/Pan Combo Test at the concentrations listed in the table below. All of the tested substances were found to have no effect on test performance.

Interfering Substance	Concentration	Interference (Yes/No)
Acetaminophen	20 mg/dL	No (5/5 negative)
Acetylsalicylic acid	20 mg/dL	No (5/5 negative)
Ascorbic acid	2 g/dL	No (5/5 negative)
Creatine	200 mg/dL	No (5/5 negative)

Oxalic acid	60 mg/dL	No (5/5 negative)
Caffeine	20 mg/dL	No (5/5 negative)
Gentisic acid	20 mg/dL	No (5/5 negative)
Albumin	2 g/dL	No (5/5 negative)
Bilirubin	1 g/dL	No (5/5 negative)

4) Cross-reactivity

The cross-reactivity of the K-Malaria P.f/Pan Combo Test was evaluated against pathogenic microorganisms. Each type of organisms was tested in triplicate, ten samples each, in the condition of presence and absence of the specific malarial antigens. No cross-reactivity was seen with the following microorganisms tested.

Specimens	Pf Reactivity	Pan Reactivity
HCV serum	Negative	Negative
HBsAg serum	Negative	Negative
COVID-19 serum	Negative	Negative
Dengue NS1 serum	Negative	Negative

12. Limitations

1. The test procedure and the result interpretation must be followed closely when using the test kit to ensure the most effective performance of the test.
2. The test kit is intended for *in vitro* diagnostic use by medical staff or trained personnel only.
3. The K-Malaria P.f/Pan Combo Test is a qualitative detection method. The intensity of the "P.f" and "Pan" line do not correlate with the number of antigens in the specimen.
4. Negative results indicate that the number of malarial antigens in the specimen is lower than the limit of detection (LOD) of the test.
5. The test is not intended to be used for monitoring anti-malarial therapy.
8. The HPR-2 antigens may be positive after two weeks of therapy, or no malaria parasites on the blood smear by microscopic examination.
9. The pLDH antigens may be positive for several days after anti-malarial therapy, or no malaria parasites on the blood smear by microscopic examination.

13. Suggested reading

1. Parra ME, et. al. Identification of *Plasmodium falciparum* histidine rich protein II in the plasma of humans with malaria. J. Clin. Microbiol. (1991), 29: 1629-1634
2. Beadle, C, et.al. Diagnosis of malaria detection of *Plasmodium falciparum* HRP-II antigen with rapid dipstick antigen capture assay. Lancet (1994), 343: 564-568.
3. World Health Organization Press Release (2001) May 23, "WHO and Novartis join forces to combat drug resistant malaria."
4. World Health Organization Fact Sheet (1998, Malaria, No.94
5. CDC/NIH Guidelines. Biosafety in Microbiological and Biomedical Laboratories. 2nd Edition, 1988.

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