Urine Malaria Test™ (UMT)
For in vitro diagnosis of Plasmodium falciparum malaria

Test Kit Product Instructions

INTENDED USE
The Urine Malaria Test™ (UMT) is an in vitro immunochromatographic test for the qualitative detection of specific Plasmodium falciparum (Pf) protein fragments shed in the urine of febrile malaria patients. It is intended to aid in the rapid diagnosis of malaria in febrile patients, and to support the differential diagnosis of malaria from other fever-causing conditions, using a freshly collected urine sample. Negative results must be confirmed by thin/thick smear microscopy.

The test is also intended for malaria epidemiological screening and monitoring in endemic regions where Pf is the predominant cause of clinical malaria in over 95% of cases.

The indication for use of the UMT is fever suspected of being malaria.

CAUTION: The UMT has not been evaluated for use with refrigerated, frozen or preserved urine samples. Clinical performance of the test has not been established for malaria due to non-Pf species.

REFERENCES

CONTACT INFORMATION
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www.fyodorbio.com
Email: UMT@fyodorbio.com

SUMMARY AND PRINCIPLE OF THE TEST
Malaria remains the single most deadly global health disease, with over half a million deaths annually. Children under 5 years account for over 70% of all deaths (WHO World Malaria Report, 2014). Pf malaria is the most deadly form and predominates in Africa, where it accounts for over 99% of clinical malaria.

Malaria episodes present with fever, but not all febrile conditions are caused by malaria. Effective case management requires early rapid diagnosis before treatment. Incorrect diagnosis and treatment of non-malaria febrile illness as malaria not only wastes resources, but also delays treatment for the actual cause of fever, resulting in poor outcomes for patients. In addition, it contributes to the increasing resistance of malaria parasites to medications.

Current World Health Organization malaria guidelines mandate parasitological diagnosis prior to treatment in all cases of fever. Since most malaria deaths occur within 48 hours of onset of symptoms, having the ability to accurately and rapidly diagnose malaria would (i) facilitate prompt access to antimalarial treatment within 24 hours of onset of symptoms, (ii) target treatment only to those who need it, and (iii) reduce malaria deaths.

Like traditional microscopy, current blood-based rapid diagnostic tests (bRDTs) detect malaria parasite antigens using finger-stick blood samples. Despite their relative simplicity, the integration of bRDTs into public and private sector settings, especially in primary care settings and in rural communities, has been very limited, in part due to the inherent risk of blood sampling in resource-limited settings and challenges with its multiplet format.

The UMT is a non-invasive dipstick test that uses a monoclonal antibody to target urine-excreted Pf parasite proteins or fragments thereof, to aid in the rapid parasitological diagnosis of malaria in febrile patients. The test strip consists of gold-conjugated capture antibody, as well as proprietary test and control line antibodies that are immobilized at specific individual sites on the nitrocellulose strip. The antibody reagents interact with the corresponding malaria parasite proteins or protein fragments therefrom, if present in the urine, resulting in pink to purple lines on the test strip.

REAGENTS AND MATERIAL
Materials Provided
• Urine Malaria Test™ (UMT)
• Sample Cup
• Instructions for Use

Materials Required But Not Provided
• Container for urine sample collection
• Timer or clock

TEST PROCEDURE
1. Collect patient urine in a clean dry container
2. Fill the Sample Cup (provided in the kit) with urine sample, up to the ridge
3. Remove test strip from packaging pouch
4. Dip the white end of the strip into the freshly collected urine specimen, with arrows pointing down
5. Allow strip to stand in sample for 25 minutes
6. Read the result

The result is read visually: One line indicates no Pf malaria; two lines indicate Pf malaria.

Test results are interpreted by the presence or absence of visually detectable pink to purple lines.

a. A positive test result (Pos) will include the detection of both a Test Line and a Control Line;

b. A negative test result (Neg) will include only a Control Line, indicating that the test worked but that Pf malarial antigens were not detected in the urine sample;

c. Failure of the Control line to appear (whether the Test line is visible or not) (Inv) indicates an invalid result. If an invalid result is obtained, repeat test using a new UMT strip.

Note: the intensity of the color in the test line region may vary depending on the concentration of Pf proteins present in the urine specimen. Therefore, any shade of color in the test line region should be considered positive.

STORAGE INSTRUCTIONS
1. Unopened UMT kits should be stored in a dry area at 2-30°C.
2. Shelf life: 24 months from date of manufacture.
3. Do not use the UMT beyond its expiration date.

PRECAUTIONS
1. For in vitro diagnostic use only.
2. The container for urine sample collection must be clean and dry.
3. Urine specimens are to be tested immediately after collection.
4. Test strip should remain sealed in its foil pouch until just before use.
5. Adequate lighting is required to read the test results.
6. The test strips are for single use only. Do not reuse test strips.
7. Patient samples and used UMT test strips should be safely handled and disposed of properly.
8. Do not tamper with UMT test strips.

WARNINGS
1. Read the package insert completely and carefully prior to use of the UMT. If the directions are not followed exactly, inaccurate test results may occur.
2. Perform the UMT procedure at room temperature (15-27°C).

QUALITY CONTROL
Procedural Control: The “C” (Control Line) position in a tested device can be considered an internal positive procedural control. If the sample flows and the reagents work, this pink to purple line will always appear.

External Positive and Negative Controls: Good laboratory practice recommends that positive and negative controls be run with each new shipment or lot to ensure that: (i) test reagents are working, and (ii) test is being performed correctly.

For training purposes, it is recommended that all first-time users of the test perform external control testing prior to running patient samples.

For positive control, a pool of 3 - 5 urine samples from confirmed malaria positive individuals can be used.

For negative control, a pool of 3 - 5 urine samples from confirmed malaria negative individuals can be used.

For further information or assistance, please contact Technical Services
(Phone: +1-443-552-0437; UMT@fyodorbio.com) or your distributor.
EXPECTED RESULTS
The rate of positive results found in malaria testing is dependent on many factors including the method of specimen collection, the test method used, geographic location, and the disease prevalence in specific localities. Over the past 10 years, average malaria infection prevalence has declined 48% in children aged 2–10, from 26% to 14% in 2013, and the number of malaria infections at any one time dropped 26%, from 173 million to 128 million in 2013 (WHO World Malaria Report, 2014). Thus, in most malaria endemic settings, more than 50% of all fevers are actually NOT due to malaria, so confirmed diagnosis is even more critical to guide prompt targeted case management. Without confirmed diagnosis, a lot of people without malaria may be wrongly treated for the disease, while the actual cause of fever is misdiagnosed and not treated.

PERFORMANCE CHARACTERISTICS
Clinical Performance (Sensitivity & Specificity)
The clinical performance of the UMT for the diagnosis of P. falciparum malaria was evaluated in a pivotal clinical trial conducted at 6 primary healthcare centers in a malaria-endemic area during rainy and dry seasons, which are periods of high and low malaria transmission. Matched urine and fingerstick blood samples from 1691 participants presenting with fever or history of fever within the previous 48 hours were collected and tested using the UMT, blood-based RDT (bRDT), and thick/thin smear microscopy, respectively. The overall agreement of the UMT results compared to thick/thin smear microscopy was used to establish the diagnostic performance (sensitivity, specificity, Positive Predictive Value [PPV], Negative Predictive Value [NPV], Likelihood Ratio Positive [LRP], Likelihood Ratio Negative [LRN] and Receiver-Operator Characteristic [ROC] curve) of the UMT.

Of the more than half a million deaths from malaria per year, 78% occur in children aged under 5 years. Using microscopy as the gold standard, the UMT performance among febrile children under 5 years of age (but older than 2 years) showed sensitivity of 93% (95% CI: 80, 98) and specificity of 83% (95% CI: 75, 89).

Since the indication for use of the UMT is fever suspected of being malaria, overall, the sensitivity among all febrile study participants was 85% (95% CI: 79, 89), and the specificity was 84% (95% CI: 80, 88).

The area under the ROC curve of the UMT (0.84 [95% CI: 0.82, 0.87]) was not significantly different from those of the bRDT comparator (0.86 [95% CI: 0.85, 0.89]), indicating that the tests do not differ in overall performance.

A community study of 228 “apparently healthy” participants was performed in two community locations to evaluate the performance of the UMT for broader clinical and epidemiological purposes. Using microscopy as the gold standard test, the specificity of the UMT was 94% [95% CI: 90, 97] and the negative predictive value of the UMT was 90%.

Study participants reporting fever, headache, chills, or body ache were more likely to test positive by the UMT than participants who did not report these symptoms, and participants receiving analgesics were also more likely to test positive by the UMT. Not surprisingly, participants receiving artemisinin-combination therapy were significantly less likely to test positive by the UMT.

Interference from Endogenous Urine Components
Leukocytes: Greater than 15 leukocytes/µl in the urine was associated with a significantly higher UMT specificity (p=0.010).

Hematuria was associated with lower specificity of the UMT (p=0.001).

Urobilinogen: UMT specificity was lower among participants with urobilinogen concentrations above 1 mg/dL of urine than in those with urobilinogen levels below 1mg/dL (p<0.001).

Elevated levels of other endogenous compounds tested (i.e. glucose, bilirubin, ketone, specific gravity, pH, protein and nitrite) in urine were not associated with statistically significant differences in the sensitivity and specificity of the UMT.

Interference from Unrelated Medical Condition
Individuals with detectable Rheumatoid Factor (RF+) and microscopically negative for malaria were tested for malaria using the UMT. All RF+ participants were negative by the UMT, resulting in a specificity of 100% (95% CI: 78%, 100%).

REPRODUCIBILITY STUDY
To evaluate test reproducibility/repeatability when performed by different individuals, two product lots used in the pivotal clinical trial were tested by three different operators, on three different days. Ten (10) strips from each of the two lots were randomly selected and tested on a panel of ten (10) clinical patient urine samples – 2 negatives, 4 low parasitemia positives (200-1999 parasites/µl), and 4 high parasitemia positives (≥2000 parasites/µl). Each operator was blinded to the results of others. The concordance among the operators was 100%.

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