**PURPOSE**

The BinaxNOW® Malaria Test is an in vitro immunochromatographic assay for the qualitative detection of Plasmodium antigens circulating in human venous and capillary EDTA whole blood of individuals with signs and symptoms of malarial infection. The test targets the histidine-rich protein II (HRPII) antigen specific to Plasmodium falciparum (P.f.) and a pan-malarial antigen (Aldolase) common to all four malaria species capable of infecting humans - P. falciparum, P. vivax (P.v.), P. ovale (P.o.), and P. malariae (P.m.). It is intended to aid in the rapid diagnosis of human malaria infections and to aid in the differential diagnosis of Plasmodium falciparum (P.f.) infections from other less virulent malarial infections. Negative results should be be confirmed by thin / thick smear microscopy or repeat RDT.

Clinical performance has not been adequately established for P. ovale (P.o.) and P. malariae (P.m.). The user must establish performance characteristics of this test with these Plasmodium species.

**PRINCIPLE**

The BinaxNOW Malaria Test uses monoclonal antibodies to detect *Plasmodium falciparum* antigen and pan-malarial antigen in venous and capillary whole blood specimens. These antibodies are immobilized on a membrane support as three distinct lines and are combined with a sample pad impregnated with visualizing particles conjugated to control and anti-malaria antibodies to create a test strip. This test strip is mounted in a book-shaped, hinged test device, along with wash and absorbent pads, intended to aid in the clearing of the membrane when the device is closed.

To perform the test, whole blood is applied to the sample pad. Malarial antigen present in the sample reacts to bind the anti-malaria conjugated antibody. Reagent A is added to the bottom of the test strip and allows the antigen-conjugate complexes to migrate along the test strip, where they are captured by the immobilized antibodies, forming the Test Line(s). Immobilized control antibody captures control conjugate, forming the Control Line. Once the blood sample has migrated the length of the test strip, the device is closed, allowing Reagent A that has been added to the wash pad to clear the test strip of excess blood.

Test results are interpreted by the presence or absence of visually detectable pink-to-purple colored lines. A positive test result, read in 15 minutes, will include the detection of both a Test Line (or Test Lines) and a Control Line. A negative test result, read in 15 minutes, will produce only a Control Line, indicating that malarial antigens were not detected in the sample. A failure of the Control Line in appearance, whether the Test Line(s) is present or not, indicates an invalid result.

**SAMPLE**

# EDTA Whole Blood specimen from fingerstick or venipuncture collection is the only acceptable specimen.

## **Venous Whole Blood**:Collect by standard venipuncture procedure using an EDTA blood collection container.

### Test samples as soon as possible. If the test cannot be performed immediately, the blood sample may be stored up to 3 days at 2°-30°C. Before testing, ensure samples are at room temperature.

## **Capillary sample**: Cleanse area with a sterile wipe or pad and dry. Use a lancet to puncture the skin and collect the blood directly into the EDTA capillary tube provided in the test kit. Fill the entire capillary tube. Testing must be performed immediately on this sample so transport to lab after collection is not acceptable.

## NOTE: Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens.

**MATERIALS**

# BinaxNOW® Malaria RDT Kit (PN 665-000): Store the BinaxNOW Malaria Test kit at 2-37°C. The BinaxNOW Malaria Test and reagents are stable until the expiration dates marked on their outer packaging and containers when stored as specified. They contain:

## Test devices are a cardboard, book shaped, hinged test device containing the test strip and several wash pads. Open pouch immediately prior to testing.

## Bottle Reagent A: a tris buffer containing detergent and sodium azide.

# BinaxNOW® Malaria Positive Control Kit (PN 665-010): lyophilized recombinant antigen control containing a mixture of HRP II and a pan-malarial protein (Aldolase) in a protein buffer with stabilizers and sodium azide as a preservative.

## Reconstitute lyophilized control with 500ul deionized water.

## Ensure complete dissolution of product, then vortex to ensure complete mixing.

## Aliquot the reconstituted control immediately into the provided cryovials by adding 25ul into each of the vials provided. Freeze the aliquots at < -20oC. Frozen control is good for the number of months printed on the cardboard vial holder if maintained at appropriate temperatures (Original lot stable for 18 months). Do not refreeze reconstituted controls.

## Using the negative control EDTA sample add 100ul of the EDTA blood to the positive control vial once thawed. Vortex prior to using to assure proper mixing.

# Negative Control: A presumed malarial negative EDTA sample less than 3 days old is used as a negative control.

# Pipettes and tips capable of delivering 15ul to 500ul of whole blood

# Deionized water

# Vortex

# Timer

**QUALITY CONTROL**

# Positive and negative external controls should be performed to assure that each lot and new shipment is performing as expected prior to patient use.

## Positive Control should generate pink to purple visible lines (even if very faint) on the Control line and both the T1 and T2 lines.

## Negative control should generate a single pink to purple visible line on the C line only.

# A procedural control line (C) is built into each run and must be positive on the cassette for the testing to be valid. If the C line does not appear the test is invalid even if the other lines (T1 and T2) appear visible.

## For daily control, the manufacturer recommends that you record these controls for each test run.

**PROCEDURE**

# Assure that all samples are warmed to room temperature prior to use.

## If using a venous blood sample, prime the pipette tip by drawing up sample and expelling it a couple of times. Then **slowly** add 15 μl of blood to the bottom half of the **PURPLE** sample pad.

## There is a white pad immediately below the purple sample pad. Hold the Reagent A bottle vertically and add two (2) free-falling drops of Reagent A to this white pad. Allow the first drop to absorb into the pad before adding the second drop. Do not add Reagent A directly to the purple pad.

## Allow the blood sample to run up the full length of the test strip. **Do not** allow the blood to run into or under the absorbent pad at the top of the strip, as doing so will hinder optimal washing (clearance) of the test strip.

### Note: If blood flow up the test strip appears to stall or is less than halfway up the strip after one (1) minute, add one (1) additional drop of Reagent A to the white pad at the bottom of the test strip (below the sample pad where the blood was added).

## Just before the blood sample reaches the base of the white absorbent pad located at the top of the test strip, **SLOWLY** add four (4) free-falling drops of Reagent A to the wash pad on the top left-hand side of the test device, **allowing each drop to absorb into the pad before adding the next**. Note that the third and fourth drops may not completely absorb into the pad.

## When the sample just reaches the base of the white absorbent pad at the top of the test strip, remove the adhesive liner from the right edge of the device, and close the device. This allows the Reagent A to wash (clear) the blood sample off the test strip. To ensure good device closure and test flow, press very firmly along the entire edge to the right of the result window

## Read the test result through the viewing window 15 minutes after closing the test device. Results read before or after 15 minutes may be inaccurate.

**INTERPRETATION OF RESULTS**

# Valid Test Results: The Control line (C) will appear on all valid tests and, when it is present, test results are interpreted as follows. Note that the appearance of any Test Line, even when very faint, indicates a positive result.

# T1 POSITIVE: Positive result for *P. falciparum* (P.f).

# T2 POSITIVE: Positive result for *P. vivax* (P.v.) or *P. malariae* (P.m.) or *P. ovale* (P.o).

# T1 + T2 Positive: Positive results for *P. falciparum* (P.f.). In some cases the appearance of both the T1 and T2 Lines may indicate a mixed infection of P.f. with another species.

# No T1 or T2 Lines: Negative result (no malaria antigens were detected).

# Invalid and/or Uninterpretable Test Results: The test is invalid if the Control (C) Line does not appear, whether a Test Line(s) is present or not.

# The test is uninterpretable if the background color hinders reading of the test result at 15 minutes. Invalid or uninterpretable tests can occur due to improper sample or Reagent A addition.

**REPORTING RESULTS**

# The Malaria BinaxNOW® Rapid Detection Test is used for preliminary results only. It is a qualitative test only looking at the presence of absence of malarial antigens.

# Those samples that test as negative should be reported as: N for Negative. A result comment “Presumptive negative for malaria antigens. Malaria antigen in the sample may be below the detection limit of the test. Negative results should be confirmed by repeat testing if malarial infection is suspected” will be automatically appended to the result.

# Those samples that test as positive should be reported as follows: P for Positive which will automatically append a comment that states the sample will be reflexed to the Blood Parasite Screen where the percent parasitemia and final speciation will be determined. An additional canned test is appended above this comment depending on which test lines are positive:

## **HT1 when T1 Positive**: “Positive for *P. falciparum* protein antigens.

## **HT2 when T2 Positive:** “Positive for malaria protein antigen, representing *P. vivax* or *P.* *malariae* or *P. ovale* or a mix of these.

## **HT12 when T1 and T2 Positive:** “Positive for *P. falciparum* protein antigen or rarely mixed infections.

**LIMITATIONS**

# A negative test result does not exclude infection with malaria, particularly at low levels of parasitemia. Therefore, the results obtained with the BinaxNOW® Malaria Test should be used in conjunction with other laboratory and clinical findings to make an accurate diagnosis. As is often done in serial microscopy testing, another sample can be collected and retested.

# The BinaxNOW Malaria Test detects antigen from both viable and non-viable malaria organisms, including gametocytes and sequestered *P. falciparum* parasites. Test performance depends on antigen load in the specimen and may not directly correlate with microscopy performed on the same specimen, therefore parasitemia levels should be determined off of a properly prepared thin smear microscopically.

# Performance of the BinaxNOW Malaria Test has not been established for monitoring treatment of malaria. Residual plasmodium antigen may be detected for several days following elimination of the parasite by anti-malaria treatment.

# Analytical reactivity testing demonstrates that the pan malaria test line (T2) on the BinaxNOW test is capable of detecting all four malaria species (P.f., P.v., P.o., or P.m.) However, during clinical trials, insufficient data was generated to support clinical performance claims for the detection of P.m or P.o. Clinical performance claims for this test are made for P.f. and P.v. detection only.

# The test is not intended for use in screening asymptomatic populations.

**INTERFERENCES**

# The BinaxNOW was tested by the manufacturer for cross reactivity with 28 different microorganisms (including Babesia, Trypanosomes and several common viruses) with no cross reactivity noted.

# The BinaxNOW was tested by the manufacturer for cross reactivity with multiple anti- malarial drugs, antibiotics, and anti-inflammatory drugs with no cross reactivity noted. Note the clinical metabolites of these drugs were not tested.

# The manufacturer evaluated high levels of endogenous blood components at higher than physiological levels for hemoglobin, protein, bilirubin (conjugated and unconjugated), and triglycerides with no effect on test performance.

# Samples with positive rheumatoid factor (RF) titers may produce false positive results in the BinaxNOW Malaria Test. Rheumatoid factors are auto-antibodies, and positive RF titers are associated with acute autoimmune disorders as well as with chronic viral infectious and parasitic infections. In addition, positive RF titers are present in 1 to 4% of the general population. The BinaxNOW test has been shown to generate false positive results in samples of some individuals with positive RF titers (in manufacturer’s testing 4 of 50 known RF positive samples caused a false positive result).

# During manufacturer’s testing one sample of 29 tested gave a false positive result when testing samples containing HAMA (Human Anti-mouse Antibody).

**SENSITIVITY AND SPECIFICITY**

# Specificity in non endemic populations: The performance of the BinaxNOW test was compared to Giemsa malaria microscopy in a prospective study conducted in the eastern US in 2006-2007. One hundred (100) whole blood specimens collected from febrile patients were evaluated on the BinaxNOW test and on microscopy. All 100 samples were negative for malaria on microscopy and negative for P. fal (100% specificity) and 99 of these samples generated negative BinaxNOW test results for P.v, P.o, P.m. yielding a specificity of 99% for these species.

# Sensitivity reported by manufacturer in endemic populations based on levels of parasitemia (0.1% parasitemia is approximately equivalent to 5000/ul) has been determined as follows:

|  |  |  |
| --- | --- | --- |
| Parasitemia level (#/ul) | % Sensitivity | 95% CI |
| >5000 | 99.7% | 98-100% |
| 1000-5000 | 99.2% | 96-100% |
| 500-1000 | 92.6% | 76-99% |
| 100-500 | 89.2% | 75-97% |
| 0-100 | 53.9% | 37-70% |
| Overall | 95.3% | 93-97% |

# Analytical Reactivity has been determined by the manufacturer as follows:

|  |  |
| --- | --- |
| **Species** | **Concentration in Parasites**  **per μl Whole Blood** |
| *P. falciparum* | 310 |
| *P. vivax* | 50-500 |
| *P. ovale* | 820 |
| *P. malariae* | 50 |

**REFERENCES**:

# BinaxNOW® Malaria Package Insert, rev 5 2012/11

# Moody, Anthony. Rapid Diagnostic Tests for Malaria Parasites. Clinical Microbiology Reviews, Jan. 2002; 15: 66-78.

# RDT selection criteria, World Health Organization, Sept 2014 WHO/HTM/GMP/2014.8

# Detection of Parasite Antigens, <http://www.cdc.gov/dpdx/diagnosticProcedures/blood/antigendetection.html>

# Laboratory Diagnosis of Blood-borne Parasitic Diseases: Approved Guideline M15A , June 2000, Vol 20 No 12

PROCEDURE HISTORY

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| --- | --- | --- | --- | --- |
| Date | Written/Issued by | New/Revised/Annual Review/Discontinued | Approved Date | Approved by |
| 12/14 | L Freeman | New | 12/17/14 | L Howell MD |
| 5/15 | L Freeman | Updated reporting to reflect LIS |  |  |
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