INTENDED USE

The OnSite Filariasis IgG/IgM Combo Rapid Test is a lateral flow immunoassay for the simultaneous detection and differentiation of IgG and IgM anti-lymphatic filarial parasites (W. bancrofti and B. malayi) in human serum, plasma, or whole blood. This test is intended to be used as a screening test and as an aid in diagnosis of infection with lymphatic filarial parasites. Any reactive specimen with the OnSite Filariasis IgG/IgM Combo Rapid Test must be confirmed with alternative testing method(s).

SUMMARY AND EXPLANATION OF THE TEST

The lymphatic filariasis known as elephantiasis, mainly caused by W. bancrofti and B. malayi, affects about 120 million people over 80 countries 1,2. The disease is transmitted to humans by the bites of infected mosquitoes within which the microfilariae sucked from an infected human subject develop into third-stage larvae. Generally, repeated and prolonged exposure to infected larvae is required for establishment of human infection.

The definitive parasitologic diagnosis is the demonstration of microfilariae in blood samples 3. However, this gold standard test is restricted by the requirement for nocturnal blood collection and lack of adequate sensitivity. Detection of circulating antigens is commercially available. Its usefulness is limited for W. bancrofti 4. In addition, microfilariaemia and antigenemia develop from months to years after exposure.

Antibody detection provides an early means to detect filarial parasite infection. Presence of IgM to the parasite antigens suggest current infection, whereas, IgG corresponds to late stage of infection or past infection 5,6. Furthermore, identification of conserved antigens allows ‘pan-filaria’ test to be applicable. Utilization of recombinant proteins eliminates cross-reaction with individuals having other parasitic diseases 7. The OnSite Filariasis IgG/IgM Combo Rapid Test uses conserved recombinant antigens to simultaneously detect IgG and IgM to the W. bancrofti and B. malayi parasites without the restriction on specimen collection.

TEST PRINCIPLE

The OnSite Filariasis IgG/IgM Combo Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing recombinant W. bancrofti and B. malayi common antigens conjugated with colloidal gold (Filariasis conjugates) and rabbit IgG-gold conjugates, 2) a nitrocellulose membrane strip containing two test bands (T1 and T2 bands) and a control band (C band). The T1 band is pre-coated with monoclonal anti-human IgM for the detection of IgM anti-W. bancrofti and B. malayi, T2 band is pre-coated with reagents for the detection of IgG anti-W. bancrofti and B. malayi, and the C band is pre-coated with goat anti rabbit IgG.

When an adequate volume of test specimen is dispensed into the sample well of the cassette, the specimen migrates by capillary action across the cassette. W. bancrofti or B. malayi IgM antibodies if present in the specimen will bind to the Filariasis conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-human IgM antibody, forming a burgundy colored T1 band, indicating a W. bancrofti or B. malayi IgM positive test result.

W. bancrofti or B. malayi IgG antibodies if present in the specimen will bind to the Filariasis conjugates. The immunocomplex is then captured by the pre-coated reagents on the membrane, forming a burgundy colored T2 band, indicating a W. bancrofti or B. malayi IgG positive test result.

Absence of any T bands (T1 and T2) suggests a negative result. The test contains an internal control (C band) which should exhibit a burgundy colored band of the immunocomplex of goat anti rabbit IgG/rabbit IgG-gold conjugate regardless of the color development on any of the T bands. Otherwise, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Positive Control (1 vial, red cap, 1 mL, Cat # R0151-P)
2. Negative Control (1 vial, green cap, 1 mL, Cat # R0151-N)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or Timer

MATERIALS REQUIRED AND AVAILABLE FOR PURCHASE

For in vitro Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not open the sealed pouch, unless ready to conduct the assay.
3. Do not use expired devices.
4. Bring all reagents to room temperature (15°C -30°C) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolized blood specimen for testing.
7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
10. Dispose of all specimens and materials used to perform the test as biohazardous waste.
11. Handle the Negative and Positive Control in the same manner as patient specimens.
12. The testing results should be read within 15 minutes after a specimen is applied to the sample well or sample pad of the device. Read result after 15 minutes may give erroneous results.
13. Do not perform the test in a room with strong air flow, ie. an electric fan or strong air-conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

Consider all materials of human origin as infectious and handle them using standard biosafety procedures.

Plasma

1. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively in Vacutainer®) by vein puncture.
2. Separate the plasma by centrifugation.
3. Carefully withdraw the plasma into new pre-labeled tube.

Serum

1. Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by vein puncture.
2. Allow the blood to clot.
3. Separate the serum by centrifugation.
4. Carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2°C to 8°C if not tested immediately.

Store specimens at 2°C to 8°C up to 5 days. The specimens should be frozen at -20°C for longer storage.

Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing. Do not use samples demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference on result interpretation.

Blood

Drops of whole blood can be obtained by either finger tip puncture or vein puncture. Do not use any hemolized blood for testing.

Whole blood specimens should be stored in refrigerator (2°C-8°C) if not tested immediately. The specimens must be tested within 24 hours of collection.

ASSAY PROCEDURE

Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well prior to assay once thawed.

Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

Step 3: Be sure to label the device with specimen’s ID number.

Step 4: For whole blood test

Apply 1 drop of whole blood (about 40-50 µL) into the sample well.

Then add 1 drop (about 35-50 µL) of Sample Diluent immediately.

For serum or plasma test

Fill the pipette dropper with the specimen.

Holding the dropper vertically, dispense 1 drop (about 30-45 µL) of specimen into...
1. The appearance of any burgundy color in the T bands, regardless of intensity, must be considered as presence of the band.

Step 5: Set up timer.

Step 6: Results can be read in 15 minutes. Positive results can be visible in as short as 1 minute. Don’t read result after 15 minutes. To avoid confusion, discard the test device after interpreting the result.

**QUALITY CONTROL**

Using individual OnSite Filariasis IgG/IgM Combo Rapid Test cassettes as described in the Assay Procedure above, run 1 Positive Control and 1 Negative Control (provided upon request) under the following circumstances to monitor test performance:

1. A new operator uses the kit, prior to performing test of specimens.
2. A new test kit is used.
3. A new shipment of kits is used.
4. The temperature used during storage of the kit falls outside of 2°C-30°C.
5. The temperature of the test area falls outside of 15°C-30°C.

Expected results are as follows:

**Negative Control**

Only the C band shows color development, the two T bands (T1 and T2) show no color development.

**Positive Control**

The C band and two T bands (T1 and T2) show color development.

The appearance of any burgundy color in the T bands, regardless of intensity, must be considered as presence of the band.

**INTERPRETATION OF ASSAY RESULT**

1. **NEGATIVE RESULT:** If only the C band is present, the absence of any burgundy color in the both T bands (T1 and T2) indicates that no anti-W. bancrofti or B. malayi antibody is detected in the specimen. The result is negative.

2. **POSITIVE RESULT:**

   2.1 In addition to the presence of C band, if only T1 band is developed, the test indicates for the presence of anti-W. bancrofti or B. malayi IgM antibody. The result is positive.

   2.2 In addition to the presence of C band, if only T2 band is developed, the test indicates for the presence of anti-W. bancrofti or B. malayi IgG antibody. The result is positive.

   2.3 In addition to the presence of C band, both T1 and T2 bands are developed, the test indicates for the presence of both IgG and IgM anti-W. bancrofti or B. malayi. The result is also positive.

**LIMITATIONS OF TEST**

1. The test result does not indicate the quantity of antibodies present in the specimen. The test may not be able to detect infections with low numbers of parasites.

2. The test result may be affected by the presence of other proteins or antibodies that may interfere with the test's ability to detect anti-filarial antibodies.

3. The test result may be affected by the presence of non-specific reactions or other substances in the specimen.

4. The test result may be affected by the presence of other infections or diseases that may cause a positive result.

5. The test result may be affected by the presence of other medications or drugs that may cause a positive or negative result.

6. The test result may be affected by the presence of other test devices or materials that may cause a positive or negative result.

**REFERENCES**


