CE CTK BIOTECH SIMPLIFYING DIAGNOSTICS

REF

Catalog Number R0151C

IVD

In vitro Diagnostic

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 the 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

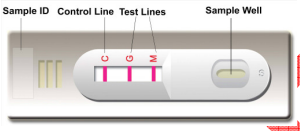
Lymphatic filariasis, commonly known as Elephantiasis, is mainly caused by *W. bancrofti and B. malayi* and affects about 120 million people across 80 countries[1,2]. The disease is transmitted to humans by the bites of infected mosquitoes within which the microfilariae sucked from an infected human subject develops 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 another commercially available diagnostic method, but its usefulness is limited to infection with *W. bancrofti*[4]. In addition, microfilaremia 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 suggests current infection, whereas, presence of IgG corresponds to late stage of infection or past infection[5]. Furthermore, identification of conserved antigens allows 'pan-filaria' tests to be applicable. Utilization of recombinant proteins eliminates cross-reaction with individuals having other parasitic diseases[6]. 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 lines (M and G lines) and a control line (C line). The M line is pre-coated with monoclonal anti-human IgM for the detection of IgM anti- *W. bancrofti and B. malayi*, the G line is pre-coated with reagents for the detection of IgG anti-*W. bancrofti and B. malayi*, and the C line 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 M line, 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 G line, indicating a *W. bancrofti or B. malayi* IgG positive test result.

Absence of any test lines (M and G) suggests a negative result. The test contains an internal control (C line) which should exhibit a burgundy colored line of the immunocomplex of goat anti-rabbit IgG/rabbit IgG-gold conjugate regardless of the color development on any of the test lines. If the C line does not develop, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
2. Plastic droppers
3. Sample diluent (1 bottle, 5 mL)
4. One package insert (instruction for use)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or Timer
2. Lancing device for whole blood test

WARNINGS AND PRECAUTIONS

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 bio-hazardous waste.
11. Handle the negative and positive controls in the same manner as patient specimens.
12. The test results should be read within 15 minutes after a specimen is applied to the sample well or sample pad of the device. Reading the results after 15 minutes may give erroneous results.
13. Do not perform the test in a room with strong air flow, i.e. an electric fan or strong air-conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test devices unopened at 2°C - 30°C**.** If stored at 2°C - 8°C, ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit over 30°C.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard bio-safety procedures.

Plasma

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

Serum

Step 1: Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by veinpuncture.
Step 2: Allow the blood to clot.
Step 3: Separate the serum by centrifugation.
Step 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. Specimens at 2°C to 8°C can be stored for up to 5 days. 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 veinpuncture. Do not use hemolized blood for testing.

Whole blood specimens should be stored in refrigeration (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.

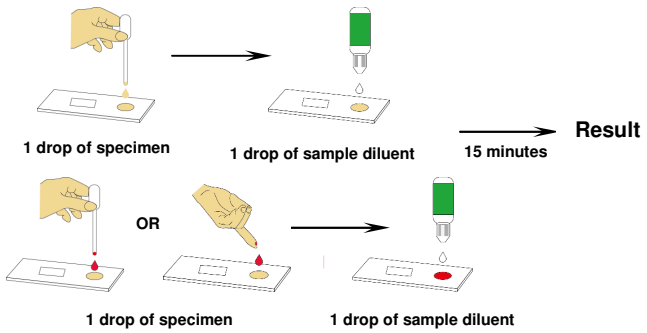
Step 2: When ready to test, open the pouch at the notch and remove the device. Place the test device on a clean, flat surface.

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

Step 4: Fill the plastic dropper with the specimen.

Holding the dropper vertically, dispense 1 drop (about 30-45 µL) of serum/plasma or 1 drop of whole blood (about 40-50 µL) into the sample well making sure that there are no air bubbles.

Immediately add 1 drop (about 35-50 µL) of Sample Diluent to the sample well.



Step 5: Set up timer.

Step 6: Results can be read in 15 minutes. Positive results can be visible in as soon as 1 minute.

Do not read the result after 15 minutes. To avoid confusion, discard the test device after interpreting the result.

QUALITY CONTROL

1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding specimen and the sample diluent. If the C line does not develop, review the whole procedure and repeat the test with a new device.

2. **External Control:** Good Laboratory Practice recommends using external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:
   a. New operator uses the kit, prior to performing the testing of specimens.
   b. A new lot of test kits is used.
   c. A new shipment of test kits is used.
   d. The temperature used during storage of the kits fall outside of 2°C - 30°C.
   e. The temperature of the test area falls outside of 15°C - 30°C.
   f. To verify a higher than expected frequency of positive or negative results.
   g. To investigate the cause of repeated invalid results.

INTERPRETATION OF ASSAY RESULT

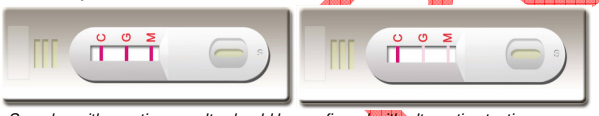
1. NEGATIVE RESULT: If only the C line is present, the absence of any burgundy color in both test lines (M and G) indicates that no anti-*W. bancrofti or -B. malayi* antibody is detected in the specimen. The result is nonreactive.

2. POSITIVE RESULT:

2.1 In addition to the presence of the C line, if only the M line is developed, the test indicates the presence of anti-*W. bancrofti or B. malayi* IgM antibody. The result is reactive.

2.2 In addition to the presence of the C line, if only the G line is developed, the test indicates the presence of anti-*W. bancrofti or B. malayi* IgG antibody. The result is reactive.

2.3 In addition to the presence of the C line, if both the M and the G lines are developed, the test indicates the presence of both IgG and IgM anti-*W. bancrofti or B. malayi*. The result is also reactive.



Samples with reactive results should be confirmed with alternative testing method(s) and clinical findings before a positive determination is made.

3. INVALID: If no C line is developed, the assay is invalid regardless of any burgundy color in the test lines as indicated below. Repeat the assay with a new device.

PERFORMANCE CHARACTERISTICS

1. Clinical Performance For IgM Test

24 samples from patients with acute lymphatic filariasis and 200 samples collected from a non-filariasis region were tested by the *OnSite* Filariasis IgG/IgM Combo Rapid Test. Comparison for all subjects is shown in the following table:

	OnSite Filariasis IgG/IgM Combo Rapid Test		
Clinical Status	**Positive**	**Negative**	**Total**
Acute filariasis	23	1	24
Negative	0	200	200
Total	23	201	224

Relative Sensitivity: 95.8%; Relative Specificity:100%; Overall agreement: 99.6%

2. Clinical Performance For IgG Test

26 samples from patients with chronic lymphatic filariasis and 200 samples collected from a non-filariasis region were tested by the *OnSite* Filariasis IgG/IgM Combo Rapid Test. Comparison for all subjects is shown in the following table:

	OnSite Filariasis IgG/IgM Combo Rapid Test		
Clinical Status	**Positive**	**Negative**	**Total**
Chronic filariasis	24	2	26
Negative	0	200	200
Total	24	202	226

Relative Sensitivity: 92.3%; Relative Specificity:100%; Overall agreement: 99.1%

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Assay Result sections must be followed closely when testing for the presence of antibodies to filarial parasites in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *OnSite* Filariasis IgG/IgM Combo Rapid Test is limited to the qualitative detection of antibodies to *W. bancrofti and B. malayi* in human serum, plasma or whole blood. The intensity of the test line does not have a linear correlation with the antibody titer in the specimen.
3. A nonreactive result for an individual subject indicates absence of detectable *W. bancrofti and B. malayi* antibodies. However, a nonreactive test result does not preclude the possibility of exposure to *W. bancrofti and B. malayi*.
4. A nonreactive result can occur if the quantity of *W. bancrofti and B. malayi* antibodies present in the specimen is below the detection limits of the assay or the antibodies that are detected are not present during the stage of disease in which a sample is collected.
5. If the symptoms persist while the result from *OnSite* Filariasis IgG/IgM Combo Rapid Test is nonreactive, it is recommended to re-sample the patient few days later or test with an alternative test method such as ELISA.
6. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect expected results.
7. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

1. Lymphatic filariasis: the disease and its control. Fifth report of the WHO Expert Committee on Filariasis. WHO Tech Rep Ser 1992; 281-871.
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3. Eberhard ML, Lammie PJ. Laboratory diagnosis of filariasis. Clin. Lab Med 1991; 11:977-1010.
4. More SJ, Copeman DB. A highly specific and sensitive monoclonal antibody-based ELISA for the detection of circulating antigen in bancroftian filariasis. Trop Med Parasitol 1990; 41:403-406.
5. Lammie PJ, Weil G, et al: Recombinant antigen-based antibody assays for the diagnosis surveillance of lymphatic filariasis-a multiplecenter trial. Flaria Jornal 2004: 3: 9-18.
6. Baskar LK, Srikanth TR, et al: Development and evaluation of a rapid flow-through immunofiltration test using recombinant filarial antigen for diagnosis of brugian and bancroftian filariasis. Microbiol Immunol. 2004: 48: 519-25.

Index of CE Symbols

	Consult instructions for use	IVD	For *in vitro* diagnostic use only		Use by
REF	Catalog #	LOT	Lot Number	Σ N	Tests per kit
30°C 2°C	Store between 2-30°C	EC REP	Authorized Representative		Do not reuse
	Manufacturer		Date of manufacture		

CTK Biotech, Inc.
10110 Mesa Rim Road
San Diego, CA 92121,USA
Tel: 858-457-8698
Fax: 858-535-1739
E-mail: info@ctkbiotech.com

EC REP **MDSS GmbH**
Schiffgraben 41, 30175 Hannover, Germany

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English version