TRUSTline Filariasis IgG/IgM Rapid Test - Cassette

for the simultaneous detection and differentiation of IgG and IgM antibodies to lymphatic filarial parasites in serum, plasma or whole blood.

INTENDED USE

The Filariasis IgG/IgM Rapid Test is a lateral flow immunoassay for the simultaneous detection and differentiation of IgG and IgM antibodies to lymphatic filarial parasites (*W. Bancrofti and B. Malayi)* in human serum, plasma or whole blood. It is intended to be used by professionals as a screening test and provides a preliminary test result to aid in the diagnosis of infection with lymphatic filarial parasites.

Any use or interpretation of this preliminary test result must also rely on other clinical findings and the professional judgment of the health care providers. Alternative test method(s) should be considered to confirm the test result obtained by this device.

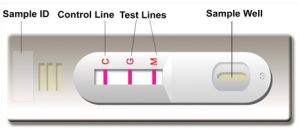
SUMMARY AND EXPLANATION OF THE TEST

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 microflariae 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 microflariae 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 Filariasis IgG/IgM 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 Filariasis IgG/IgM 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 a control antibody conjugated with colloidal gold, 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 to *W. bancrofti and B. malayi*, the G line is pre-coated with reagents for the detection of IgG to *W. bancrofti and B. malayi*, and the C line is pre-coated with a control antibody.

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. Anti-*W. bancrofti* or *anti-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 anti-*W. bancrofti* or anti-*B. malayi* IgM positive test result.

Anti-*W. bancrofti* or anti-*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 an anti-*W. bancrofti* or anti-*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 the control antibodies 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 (5 mL/bottle)
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 (15-30°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 hemolyzed 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 15 minutes after a specimen is applied to the sample well of the device. Any results interpreted outside of the 15 minute window should be considered invalid and must be repeated.
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 device unopened at 1-30°C. If stored at 2-8°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 to temperatures above 30°C.

SPECIMEN COLLECTION AND HANDLING

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

Plasma/Serum

Step 1: Collect blood specimen into collection tube containing EDTA, citrate or heparin for plasma or collection tube containing no anticoagulants for serum by venipuncture.

Step 2: To make plasma specimen, centrifuge collected specimens and carefully withdraw the plasma into a new pre-labeled tube.

Step 3: To make serum specimen, allow blood to clot, then centrifuge collected specimens and carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2-8°C, if not tested immediately. The specimens can be stored at 2-8°C for 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 with result interpretation.

Whole Blood

Step 1: Drops of whole blood can be obtained by either fingertip puncture or venipuncture. Collect blood specimen into a collection tube containing EDTA, citrate or heparin. Do not use hemolyzed blood for testing.

Whole blood specimens should be stored in refrigeration (2-8°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.

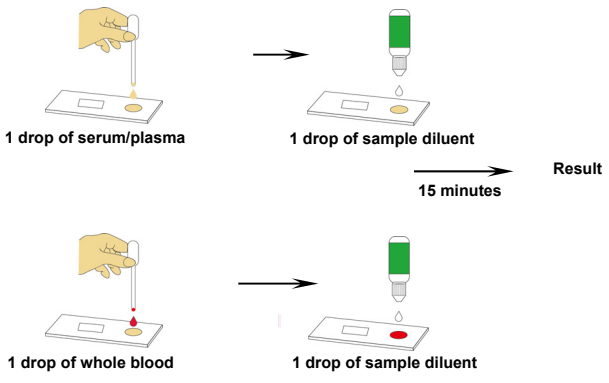
Step 2: When ready to test, open the pouch at the notch and remove the device. Place the test device on a clean, flat surface.

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 with the bottle positioned vertically.



Step 5: Set up timer.

Step 6: Results can be read at 15 minutes. Positive results can be visible in as soon as 1 minute. Negative results must be confirmed at the end of the 15 minutes only. ***Any results interpreted outside of the 15 minute window should be considered invalid and must be repeated. Discard used devices after interpreting the result following local requirements governing the disposal of devices.***

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. 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 1-30°C.
   e. The temperature of the test area falls outside of 15-30°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* IgG or IgM antibody is detected in the specimen. The result is non-reactive or negative.

2. **POSITIVE RESULT**:

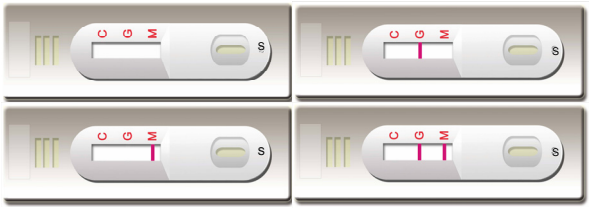
2.1 In addition to the presence of the C line, if only the M line develops, the test indicates the presence of anti-*W. bancrofti* or anti-*B. malayi* IgM antibody. The result is IgM reactive or positive.

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

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

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

3. **INVALID:** If no C line develops, 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 Filariasis IgG/IgM Rapid Test. Comparison for all subjects is shown in the following table:

	Filariasis IgG/IgM 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 Filariasis IgG/IgM Rapid Test. Comparison for all subjects is shown in the following table:

	Filariasis IgG/IgM 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 IgG and IgM antibodies to filarial parasites in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The Filariasis IgG/IgM Rapid Test is limited to the qualitative detection of IgG and IgM 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 anti-*W. bancrofti* and anti-*B. malayi* IgG and IgM 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 Filariasis IgG/IgM Rapid Test is nonreactive, it is recommended to 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.
2. Michael E, Bundy DAP, Grenfell BT. Re-assessing the global prevalence and distribution of lymphatic filariasis. Parasitology 1996; 112:405-428.
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 Symbols

Consult instructions for use	REF Catalogue number	Use-by date
IVD For *in vitro* diagnostic use only	LOT Batch code	Tests per kit
Temperature limit 1-30 °C	Do not re-use	Keep dry
Manufacturer	Date of manufacture	European Conformity
NON STERILE If device is non-sterile	Warnings / Precautions	EC REP Authorized Representative
Do not use if package is damaged	Keep away from sunlight	