# FILARIASIS CARD

**For in *Vitro* diagnostic use only**

**Immunochromatographic test on format CARD for the simultaneous detection and differentiation of IgG and IgM anti-lymphatic filarial parasites in human serum, plasma or whole blood**

## I. INTENDED USE

The FILARIASIS CARD Mascia Brunelli 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).

## II. SUMMARY AND EXPLANATION OF THE TEST

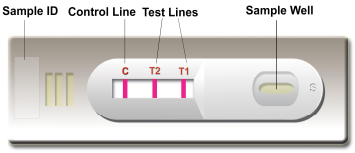
The lymphatic filariasis known as Elephantiasis, mainly caused by W. bancrofti and B. malayi, affects about 120 million people over 80 countries. The disease is transmitted to humans by the bites of infected mosquitoes within which the microflariae sucked from an infected human subject develops into 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. 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. 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 suggest current infection, whereas, IgG corresponds to late stage of infection or past infection. 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. The FILARIASIS CARD Mascia Brunelli uses conserved recombinant antigens to simultaneously detect IgG and IgM to the W. bancrofti and B. malayi parasites without the restriction on specimen collection.

## III. PRINCIPLE OF THE TEST

The FILARIASIS CARD Mascia Brunelli 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 colloid 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.

## IV. REAGENTS AND MATERIALS PROVIDED

- Each kit contains 25 test devices, each sealed in a foil pouch with three items inside:
  - One cassette device
  - One plastic dropper
  - One dessicant.
- Sample diluent (1 bottle, 3.0 ml)
- Instruction for use (1 item)

## V. MATERIALS REQUIRED BUT NOT PROVIDED

- Clock or timer
- Lancing device for whole blood test.

## VI. PRECAUTIONS

1. For *in vitro* and professional use only.
2. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
3. Do not open the sealed pouch, unless ready to conduct the assay.
4. Do not use expired devices.
5. Bring all reagents to room temperature (15°C -30°C) before use.
6. Do not use the components in any other type of test kit as a substitute for the components in this kit.
7. Do not use hemolized blood specimen for testing.
8. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
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. 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.
12. Do not perform the test in a room with strong air flow, ie. an electric fan or strong air-conditioning.

## VII. REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test devices unopened at 2°C -30°C. The positive and negative controls should be kept at 2°C -8°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.

## VIII. SPECIMEN COLLECTION AND HANDLING

Consider any 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 veinpuncture.
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 veinpuncture.
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 veinpuncture. Do not use any 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.

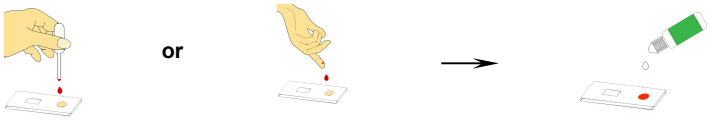
## IX. ASSAY PROCEDURE

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.

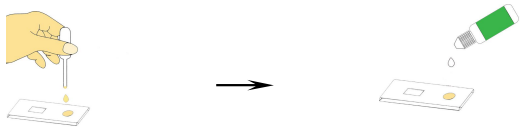
Then add 1 drop (about 35-50 µL) of Sample Diluent immediately.




**1 drop of whole blood** **1 drop of sample diluent**

**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 the sample well making sure that there are no air bubbles.

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



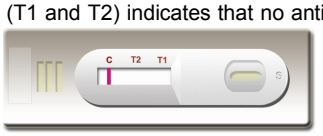

**1 drop of sample** **1 drop of sample diluent** **15 minutes**

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.**

## X. INTERPRETATION OF ASSAY RESULTS

1. **NEGATIVE**: 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**:

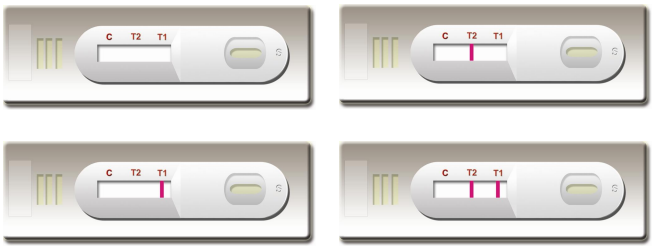
**2.1** In addition to the presence of C band, if only T1 band is developed, the test indicates for the presence of anti-W. bancrofti or B. malayi IgM antibody. The result is positive.


**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.


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

**3. NON VALIDO**: If no C band is developed, the assay is invalid regardless of any burgundy color in the T bands as indicated below. Repeat the assay with a new device.




## XI. PERFORMANCES CHARACTERISTICS

### 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 CARD Mascia Brunelli. Comparison for all subjects is showed in the following table:

| | FILARIASIS Card | | |
|---|---|---|---|
| **Clinical Status** | **Positive** | **Negative** | **Total** |
| **Acute Filariasis** | 23 | 1 | 24 |
| **Negative** | 0 | 200 | 200 |
| **Total** | 23 | 201 | 224 |

Relative Sensitivity: 95.8% , Relative Specificity: 100.0%, Overall agreement 99.6%

### Clinical Performances 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 CARD Mascia Brunelli. Comparison for all subjects is showed in the following table:

| | FILARIASIS Card | | |
|---|---|---|---|
| **Clinical Status** | **Positive** | **Negative** | **Total** |
| **Chronic filariasis** | 24 | 2 | 26 |
| **Negative** | 0 | 200 | 200 |
| **Total** | 24 | 202 | 226 |

Relative Sensitivity: 92.3% , Relative Specificity: 100.0%, Overall agreement 99.1%

## XII. LIMITATIONS OF THE TEST

1. The Assay Procedure and the Test Result Interpretation must be followed closely when testing 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 FILARIASIS CARD Mascia Brunelli 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 band does not have linear correlation with the antibody titer in the specimen.
3. A negative result for an individual subject indicates absence of detectable W. bancrofti and B. malayi antibodies. However, a negative test result does not preclude the possibility of exposure to W. bancrofti and B. malayi.
4. A negative 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. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
6. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

## XIII. 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

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|---|---|---|---|---|---|---|---|---|---|---|---|
| IVD | In Vitro Diagnostic Medical Device | | Temperature limitation | LOT | Batch code (EXXX) | | Manufacturer | | Keep dry | NON STERILE | Non-sterile |
| | Consult Instructions for use | | Use by (year/month) | REF | Catalogue number | | Do not reuse | | Fragile, handle with care | | Keep away from heat |

| **CONTENT (25 tests)** | **COD. VQ85300** |
|---|---|
| Card | 25 devices |
| Sample diluent | 1 x 3.0 mL |
| Instruction for use | 1 item |

**EDMA CODE 14 70 01 90 00** CE IVD