PERFORMANCE CHARACTERISTICS

PanLF Rapid has been evaluated in many endemic country including Malaysia, Indonesia, India and Sri Lanka. Sensitivity and specificity reported are 94-100% and 99-100% respectively.

INTENDED USE

For the detection of specific IgG, antibodies to Wuchereria bancrofti, Brugia malayi and/or Brugia timori in human serum, plasma or whole blood. It is intended to be used as in vitro diagnostic test for lymphatic filariasis.

SUMMARY AND EXPLANATION OF THE TEST

Lymphatic filariasis is caused by three species of blood/tissue worms namely Wuchereria bancrofti, Brugia malayi and Brugia timori. One hundred and twenty million people in 83 countries of the world are infected with lymphatic filarial parasites, and it is estimated that more than 1 billion (20% of the world’s population) are at risk of acquiring the infection. Ninety percent of these infections are caused by Wuchereria bancrofti (http://www.filariasis.org).

The disease is transmitted by several species of mosquitoes. Infective larvae from the mosquito enter the human through the puncture wound made by the insect. The larvae then travel to lymphatic channels and lymph nodes and develop into adult worms.

Infection with Wuchereria bancrofti, Brugia malayi and Brugia timori will result in symptoms and manifestations that may include recurrent fever, lymphatic damage, renal damage, adenolymphangitis, lymphoedema, hydrocoele (only in W. bancrofti infection), elephantiasis and pulmonary disease.

Specific recombinant filarial genes have been expressed, purified and incorporated into an immunochromatography-based rapid test in a cassette format called PanLF Rapid for the detection of specific IgG, to Wuchereria bancrofti, Brugia malayi and/or Brugia timori. PanLF Rapid offers many advantages which include:

- usage of whole blood or serum/plasma as sample
- results in 15-25 minutes
- sample collected any time of the day can be used
- simple to perform
- no special equipment is needed
- results are easy to interpret
- minimal sample volume (35 µl for whole blood or 30 µl for serum/plasma)

PRINCIPLE OF THE TEST

PanLF Rapid is an indirect solid-phase immunochromatographic assay. Specific recombinant antigens are immobilized on cellulose nitrate membrane strip (at B and C; refer diagram on page 2). When the test sample is added to the sample pad, it migrates upwards. If specific antibodies are present in the test sample (blood), it forms an antibody-antigen complex with the immobilized antigen(s) at the test window zone(s). The bound antibody-antigen complexes are subsequently detected by a dye conjugated anti-human IgG, when the chase buffer is added and it migrates downward, giving a pink-purple colour. The control line (at A) contains goat anti-mouse IgG antibody which binds with the mouse anti-human IgG-dye conjugate. The control band serves as an indication of proper migration plus reagent (mouse anti-human IgG-dye conjugate) control.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Sample collection and preparation device and equipment
2. Sample dispensing apparatus such as pipettes
3. Clock or timer

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic professional use only.
2. This product insert must be strictly adhered in order to produce accurate test results. Deviations from the procedure may lead to aberrant results.
3. Keep the test cassettes sealed until use.
4. Do not use cassette if the sealed pouch is visibly damaged.
5. Do not use kit beyond the expiration date.
6. Autoclave all used and contaminated materials at 121°C, 15 p.s.i. for 30 minutes before disposal. Alternatively, decontaminate materials in 5% sodium hypochlorite solution for 30-60 minutes before disposal in biohazard waste-bags.
7. Wipe any spills of sera or plasma promptly with 1% sodium hypochlorite solution.
8. Do not reuse test device.

STORAGE AND STABILITY

Store test cassettes and chase buffer at 4-30°C, do not freeze. The test cassettes should be kept sealed until use.

SAMPLE COLLECTION AND PREPARATION

For whole blood samples, 35 µl of whole blood from the pricked finger needs to be collected and placed into the square well of the test cassette (refer to Step 1 in Assay Procedure). There are 3 methods for doing this step, namely:

a. The 35 µl whole blood can be collected from the finger using a calibrated microcapillary tube with anti-coagulant and added to the square well of the test cassette (procedure for preparing the calibrated microcapillary tube is given in the product insert)

b. Several drops of blood from the finger can be collected directly into a micro collection tube coated with anti-coagulant. Before performing the test, 35 µl of blood can be taken up using a micropipettor.

c. The 35 µl whole blood can be directly pipetted from the finger and immediately delivered into the square well, making sure that there are no air bubbles. However this method should only be performed by an experienced person to avoid pipetting of incorrect volume of blood.

Procedure for preparation of marked microcapillary tube

Since each brand of microcapillary tube has different “dead volume”, the following is a guideline that can be used to create marked microcapillary tube to deliver 35 µl of blood:

a. Pipette X µl of water (e.g. 50 µl) onto a clean glass slide.

b. Use a microcapillary tube, take up all the water (via capillary action).

c. Cover the end of the microcapillary tube with the tip of index finger.

d. Release the finger and let all the water flow out (without forcing it out) on a clean dry area of the slide.

e. With a micropipettor, measure the volume of water that was delivered onto the slide.

f. Repeat steps a to e with various X µl of water until the volume of water released onto the slide is 35 µl.

g. When the final volume of X is determined, use a new microcapillary tube to take-up X µl of water and mark the height of water level on the tube with a permanent marker.

h. This tube will then be used as a reference to mark other microcapillary tubes of the same brand.

ASSAY PROCEDURE

1. Bring test cassette and chase buffer to room temperature (if precipitates are noted in the chase buffer reagent, shake the bottle vigorously and allow it to warm up further).

2. Open the pouch by gently tearing at the notch of the pouch.

3. Label the test device with the sample name.

4. Proceed with the assay procedures as diagrammed below.
Diagrammatic Representation of Assay Procedure

Step 1:
For serum/plasma sample:
Add 30 µl of sample to square well. (In the rare event that serum/plasma is not wicking up after 2-3 minutes, add one drop of chase buffer to square well.)

For whole blood sample:
Release 50 µl of blood sample by touching the tip of the micropipette/ microcapillary tube onto the sloping wall of the square well. Allow the blood to flow from the slope to the white sample pad. Add 1 drop of chase buffer to the same well.

Serum/plasma will start wicking up the membrane. Red blood cells will wick slower. The cassette may be tapped gently on the table to facilitate the sample to flow up the membrane. Wait until the wet sample front of the serum/plasma reaches the blue line. Go to step 2.

If the sample front does not reach the blue line after waiting for approximately 3 minutes but has already reached area B, please proceed to step 2.

Step 2:
Add 3 drops of chase buffer, drop-by-drop and let it sip through the pad in between each drop. Go to step 3.

Step 3:
Pull clear tab until resistance is felt. Add 1 drop of chase buffer to the square well. Start timing. Read results within 15 minutes for serum/plasma sample. For whole blood sample, read results within 25 minutes to allow clearing of red blood cells from the membrane strip.

QUALITY CONTROL
1. Positive and negative controls are not included.
2. If the control line at position A does not become visible, the test is invalid. Positive samples will have additional coloured band(s) at positions B and/or C.

INTERPRETATION OF RESULTS
Positive
Negative
Invalid

1. Any intensity of band should be considered as presence of the band.
2. Positive for either or/and Wuchereria bancrofti, Brugia malayi and Brugia timori specific antibodies if coloured bands appear at the Control line (A) and Test line(s) at B, C or both B & C as shown in the diagram above.
3. Negative for Wuchereria bancrofti, Brugia malayi and Brugia timori specific antibodies if only the Control line (A) is visible through the viewing window.
4. Invalid if the Control line (A) is absent. If this occurs, the assay should be repeated using a new test cassette.

LIMITATIONS OF THE TEST
1. This product is designed for use with human serum, plasma or whole blood only.
2. The test is a qualitative assay and is not for quantitative determination of antibodies concentration levels. The intensity of the band does not have linear correlation with the antibody titer of the specimen.
3. The results obtained should not be the sole determinant for clinical decision.

WARRANTY AND LIMITED LIABILITY
The performance characteristics stated were obtained by using the assay procedure in this insert. Failure to follow the assay procedure may derive inaccurate results. In such event, the manufacturer disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and the fitness for use.

The manufacturer will not be liable for any damage caused by misuse, improper handling and storage, non-compliance with warnings and procedures, damage caused by events occurring after the product is released, failure to ensure the product is in proper condition before use, or any warranty given by independent distributor.

PATENT INFORMATION
1. Filarial parasite polypeptides and sequences, gene sequences and uses thereof:
   - Malaysia Patent No. MY134618A
   - Indonesia Patent No. ID P 0023218
   - India Patent No. 954/MUM/2000
   - U.S. Patent No. 12/297325
   - Philippines Patent No. 1-2008-50298
2. Method to detect anti-filarial antibodies to SXP-1 recombinant antigen using immunochromatography technology:
   - U.S. Patent No. 12/297325
   - India Patent No. 8791/DELNP/2008
   - Vietnam Patent No. 1-2008-02531
   - Sri Lanka Patent No. 15154
   - Malaysia Patent No. Pi 2006 1740 (Pending)

ACKNOWLEDGEMENT
Device and methods covered under U.S. Patent 6316205 owned by Genelabs Diagnostics Pte. Ltd.

REFERENCES
2. Rohana AR, Cheah HY and Rahmah N. PanLFD-ELISA using BmR1 and BmSXP recombinant antigens for detection of lymphatic filariasis. Filarial Journal 2007; 6: 10 (http://www.filarialjournal.com/content/6/1/10)

ORDER INFORMATION
<table>
<thead>
<tr>
<th>Product Code</th>
<th>Product Name</th>
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<tr>
<td>LF-RD0101</td>
<td>PanLF Rapid test kit</td>
<td>25 tests / kit</td>
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</tbody>
</table>

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