**Title:** METHOD FOR RAPID DETECTION OF LYMPHATIC FILARIASIS

**Abstract:** There is provided by this invention a specific and sensitive diagnostic method for rapid detection of lymphatic filariasis. The method employs a combination of SXP/SPX-1 recombinant antigen, mouse monoclonal anti-human IgG4 antibody conjugated to a detection reagent and the technique of immunochromatiography.
Method for Rapid Detection of Lymphatic Filariasis

1. Technical Field of the Invention

The present invention relates to a method for rapid detection of lymphatic filariasis, particularly a method that detects anti-filarial IgG4 antibodies in a biological sample using a SXP/SXP-1 recombinant antigen and the technique of immunochromatography.

2. Background of the Invention,

Lymphatic filariasis is a parasitic and infectious tropical disease caused by a number of slender and thread-like parasitic filarial worms which invade blood circulation, lymphatics, lymph nodes and other parts of the human body.

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 and the remainder by Brugia malayi and Brugia timori. The disease has been identified by the World Health Organization (WHO) as the second leading cause of permanent and long-term disability in the world.

Lymphatic filariasis is transmitted to man by mosquitoes, which introduce a large number of infective larvae into human. The female warijis produce
microfilariae, which make their way into blood circulation and are taken up by a suitable mosquito species. On reaching the mosquito's body, the microfilariae (first stage larvae, L1 larvae) undergo several moulttings to form infective larvae (L3 larvae) which reach the blood circulation of the definitive host through wound made by the bites of the mosquitoes. Soon the infective larvae enter into the lymphatic system where they slowly mature into adult male and female worms. The male and female adult mate to produce microfilariae which find their way to peripheral blood circulation.

The clinical manifestations of lymphatic filariasis can be divided into three types:

1. Asymptomatic: these individuals are outwardly non-symptomatic, but will demonstrate lymphatics and/or renal damage if appropriately tested.

2. Acute manifestations: these include "filarial fever" or acute attacks associated with inflammation of the lymphatic flodes and channels.

3. Chronic manifestations: these arise from adult worm's damage to the lymphatic system, and include hydrocoele, lymphoedema, chyluria and elephantiasis.

The traditional or routine method to diagnose filarial infection depended on the direct demonstration of the microfilariae in blood using relatively cumbersome techniques and having to take
into account the periodicity of microfilariae in blood. This traditional method severely lacks sensitivity (25% - 40% sensitive), thus missing many positive cases. This is due to the inability of the method to detect cryptic infections (before microfilariae are produced and after microfilariae ceased to be produced), single sex infections, occult infections and low levels of microfilariae. Blood concentration techniques such as the Knoti's method and membrane filtration increases the sensitivity of detection but are usually not performed because they require venous blood taking. Polymerase chain reaction (PCR) -based detection methods are very sensitive to detect low levels of microfilariae, however it is not suitable for detection of cryptic, occult or single sex infections.

In year 1998, WHO initiated a Global Program for Elimination of Lymphatic Filariasis (GPELF). The main aim of this program is to eliminate lymphatic filariasis as a public health problem by reducing the level of the infection in endemic populations to a point whereby active transmission no longer occurs. The main approach that is being taken is to provide mass drug treatment once yearly to the entire 'at risk' population for a period of 4-6 years. Availability of diagnostic tools is one of the important factors for success of this program. The tools are needed for accurate mapping of endemic areas, for monitoring activities, certification of elimination and surveillance activities post-elimination. For bancroftian filariasis, a rapid
antigen test is commercially available for mapping and monitoring activities, but a rapid test based on antibody detection is also needed, particularly for the certification and post-elimination surveillance phases of the program.

SXP or SXP-I gene (Genbank accession No. M98813) was previously identified by immnoscreening of Brugia malayi cDNA library with immune sera from microfilariae positive patients with brugian and bancroftian filariasis (Dissanayake S., Xu M., Piessens WF. A cloned antigen for serological diagnostic of Wuchereria bancrofti microfilariae with daytime blood samples. MoI Biochem parasitol 1992: 256-26). The Brugia malayi-derived recombinant protein (JBM-SXP-I) was reported to be successful in identifying 83% (64/72) of bancroftian filariasis patients when tested with IgG4-ELISA (Chandrashekar R., et al Molecular cloning of Brugia malayi antigens for diagnosis of lymphatic filariasis. MoI Biochem Parasitol 1994:64 (2): 262-271). With this method (IgG4-ELISA), Wuchereria bancrofti-derived protein homologue of SXP-I (Wb-SXP-I) was reported to detect 100% (72/72) of the Wuchereria bancrofti infected patients. (Rao KV., et al The Wuchereria Bancroft! orthologue of Brugia malayi SXP-I and the diagnosis of bancroftian filariasis. MoI Biochem Pa.rasx.tol 2000; 107 (1): 71-80).

Although the expression products of SXP/SXP-1 gene has been reported over the years and proven to successfully detect lymphatic filariasis in infected
patients, the SXP/SXP-1 gene was applied for the development of tests using ELISA format, which requires several hours to perform. There is no report of the application of the expression products of SXP/SXP-1 gene using the immunochromatography technique for rapid detection of filariasis. Thus, there remains a need in the art for a rapid detection of lymphatic filariasis that employs the SXP/SXP-1 gene and the technology of immunochromatography for the reason discussed above.

3. Summary of the Invention

It is a general object of the present invention to provide a diagnostic method, which is capable of detecting lymphatic filariasis in infected patients by detecting anti-filarial IgG4 antibodies in a biological sample.

It is also an object of the present invention to provide a specific and sensitive diagnostic method, which is capable of detecting anti-filarial IgG4 antibodies in a biological sample by using SXP/SXP-1 recombinant antigen and the technique of immunochromatography.

Another object of the present invention is to provide a simple and rapid diagnostic kit employing the method outlined above that can be performed by untrained personnel in a minimum amount of time.

These and other objects of the present invention
are achieved by,

A method for rapid detection of lymphatic filariasis in a biological sample, comprising the steps of:

a) Adding a buffer to reconstitute diluted mouse monoclonal anti-human IgG4 antibody conjugated to a detection reagent prepared separately in a microwell;

b) Adding a biological sample to the sample receiving end of a chromatographic element;

c) Allowing the biological sample to flow from the sample receiving end to the reaction zone of the chromatographic element wherein SXP/SXP-1 recombinant antigen is immobilized within the reaction zone;

d) Placing the chromatographic element in (c) into the microwell containing the reconstituted mouse monoclonal anti-human IgG4 antibody conjugated to a detection reagent in (a);

e) Allowing the mouse monoclonal anti-human IgG4 antibody conjugated to a detection reagent to flow from the microwell to the reaction zone of the chromatographic element; and

f) Detecting the complex formed in (e).

A method for rapid detection of lymphatic filariasis in a biological sample, comprising the steps of:

a) Adding a biological sample to the sample receiving end of a chromatographic element;

b) Allowing the biological sample to flow from the sample receiving end to the reaction zone of the
A method for rapid detection of lymphatic filariasis in a biological sample, comprising the steps of:

a) Adding a biological sample to the sample receiving end of a chromatographic element;

b) Allowing the biological sample to flow from the sample receiving end to the reaction zone of the chromatographic element wherein SXP/SXP-1 recombinant antigen is immobilized within the reaction zone;

c) Adding a buffer to the reagent releasing end of the chromatographic element to reconstitute the dried mouse monoclonal anti-human IgG4 antibody conjugated to a detection reagent incorporated therein;

d) Allowing the mouse monoclonal anti-human IgG4 antibody conjugated to a detection reagent to flow from the reagent releasing end to the control zone of the chromatographic element wherein anti-mouse IgG antibody is immobilized within the control zone;

e) Allowing the mouse monoclonal anti-human IgG4 antibody to flow from the reagent releasing end to the reaction zone.
antibody conjugated to a detection reagent -fco further
flow from the control zone to the reaction zone of the
chromatographic element; and

Detecting the complex formed in (d) & (e).

A diagnostic kit for rapid detection of lymphatic
filariasis in a biological sample comprising a
detection device wherein the detection device
comprises a chromatographic element wherein the
chromatographic element comprises a sample receiving
end, a reaction zone and a control zone characterized
in that SXP/SXP-1 recombinant antigen is immobilized
within the reaction zone and dried mouse monoclonal
anti-human IgG4 antibody conjugated to a detection
reagent is in a separate microtiter well.

A diagnostic kit for rapid detection of lymphatic
filariasis in a biological sample comprising a
detection device wherein the detection device
comprises a chromatographic element wherein the
chromatographic element comprises a sample receiving
end, a reagent releasing end, a reaction zone and a
control zone characterized in that SXP/SXP-L
recombinant antigen is immobilized within the reaction
zone.

4. Brief Description of the Accompanying Drawings

Other aspects of the present invention and their
advantages will be discerned after studying the
detailed description in conjunction with the
accompanying drawings in which:
Figure 1 shows the plasmid map of SXP/SXP-I recombinant gene.

Figure 2 shows the SDS-PAGE profile of SXP/SXP-I recombinant protein.

Figure 3 shows the appearance of tests in a dipstick and cassette format employing the method for rapid detection of lymphatic filariasis.

5. Detailed Description of the Invention

The method for rapid detection of lymphatic filariasis in accordance with the present invention detects anti-filarial IgG antibodies in a biological sample based on a specific antibody-antigen binding reaction, which comprises a recombinant antigen, expressed by the SXP/SXP-1 gene binding to the anti-filarial IgG antibodies in the biological sample. The SXP/SXP-1 gene is a recombinant gene derived from filarial parasites *Brugia malayi*, *Wuchereria bancrofti* or *Brugia timori*.

In the present invention, SXP/SXP-1 gene was cloned from *Brugia malayi* cDNA library by established PCR cloning methodology using the following primers: Forward; 5' GTC ACT TCA TCA CTC AAT 3' and Reverse: 5' CTA TTT ATT ACT TTT TGT CG 3'. The recombinant gene was recloned into a bacterial expression vector (pPROξXHT, Life Technologies) and the His-tagged recombinant gene as shown in DNA sequence ID No.: 1.
and Figure 1 was transformed into *E. coli* TOP 10 host (Invitrogen). Any other expression vector such as prokaryote, insect or mammalian expression vector may be used in the present invention.

Expression of the SXP/SXP-1 recombinant gene was then induced with isopropyl-b-D-thiogalactos ïde (IPTG) to produce recombinant protein and followed by purifying the recombinant protein by affinity chromatography. In the purification step, AKTA Prime Purification System (Pharmacia) and chromatography column packed with Ni-WTA resin (Qiagen, USA) were employed. The protein containing fractions were pooled and passed through using spin columns (MWCO 10kDa) for buffer exchange and concentration of the recombinant protein.

SDS-PAGE analysis of the SXP/SXP-1 recombinant protein in Figure 2 shows that it has an apparent molecular weight of approximately 30 kDa. Western blot analysis of the purified recombinant protein showed that the SXP/SXP-1 recombinant antigen produced was sensitive and specific for detection of lymphatic filariasis.

IgG4 assay in an ELISA format was then developed using SXP/SXP-1 recombinant antigen and the assay was evaluated using serum samples from individuals infected with various infections and normal individuals. The results showed that the igG4 assay developed was highly sensitive and specific for detection of *Nucheneria bancrofti* infection. The
recombinant antigen also reacted with serum samples from *Brugia malayi* and *Brugia timori* patients, however the sensitivity was found to be lower.

The assay was further developed into a rapid assay that employs the SXP/SXP-1 recombinant antigen and the technique of immuno chromatography. This technology of lateral flow or immunochromatography refers to capillary flow of immunological components through an absorbent membrane to mix and sequentially separate the various components.

The rapid immunochromatography assay for detecting filariasis of the present invention includes a chromatographic element comprising three generally contiguous sections: sample receiving end, reaction zone and control zone. Chromatographic element refers to a solid matrix upon which the sample can be applied and allowed to migrate during the assay procedure. The chromatographic element particularly preferred in this invention is an absorbent nitrocellulose membrane. Other chromatographic elements that can be used include nylon and/or mixed esters. SXP/SXP-1 recombinant antigen is immobilized within the reaction zone and anti-mouse IgG antibody is immobilized within the control zone.

In one embodiment of the present invention, dried mouse monoclonal anti-human IgG4 antibody conjugated to a detection reagent is prepared separately in a microwell. In another embodiment of the present invention, the dried mouse monoclonal anti-human IgG4
antibody conjugated to a detection reagent is incorporated within the reagent releasing end of the chromatographic element. Any substance that is capable of producing a detectable signal can be used as the detection reagent conjugated to the mouse monoclonal anti-human IgG4 antibody including colloidal metallic particles such as gold and silver, colloidal non-metal particles such as selenium, tellurium and sulfur and also organic polymer latex particles. Detection reagents preferred for use in the present invention are the visually detectable coloured particles, such as colloidal metals, particularly colloidal gold.

In a first embodiment of the present invention, a buffer is added to reconstitute dried mouse monoclonal anti-human IgG4 antibody conjugated to colloidal gold in a microwell. Then, a biological sample such as blood, serum, plasma, urine or tears is introduced to the sample receiving end of an absorbent nitrocellulose membrane and is allowed to migrate laterally via capillary action towards the reaction zone of the membrane. The anti-filarial IgG4 antibodies present in the sample will bind to the SXP/SXP-1 recombinant antigen immobilized within the reaction zone, forming an antibody-antigen -Complex or immune-complex. Next, the absorbent nitrocellulose membrane is placed in the microwell containing the reconstituted mouse monoclonal anti-human IgG4 antibody conjugated to colloidal gold. The mouse monoclonal anti-human IgG4 antibody conjugated to colloidal gold absorbs through the membrane and migrates to the reaction zone and binds to the
antibody-antigen complex formed earlier thus forming a complex which comprises SXP/SXP-1 recombinant antigen, anti-filarial IgG4 antibodies and mouse monoclonal anti-human IgG4 antibody conjugated to colloidal gold. The presence of gold in the complex will result in the appearance of a red-purplish line at the location of the reaction zone indicating the presence of anti-filarial IgG4 antibodies in the sample tested. The unbound mouse monoclonal anti-human IgG4 antibody conjugated to colloidal gold from the reaction zone will further migrate to the control zone and bind with the anti-mouse IgG antibody incorporated therein, forming a red-purplish line in the control zone. This control zone serves as an internal control to ensure the stability of the gold conjugated reagent.

In a second embodiment of the present invention, a biological sample such as blood, serum, plasma, urine or tears is first introduced to the sample receiving end of the absorbent nitrocellulose membrane and is allowed to migrate laterally via capillary action towards the reaction zone of the membrane. The anti-filarial IgG4 antibodies present in the sample will bind to the SXP/SXP-1 recombinant antigen immobilized within the reaction zone, forming an antibody-antigen complex or immunocomplex. A buffer is then introduced to the reagent releasing end to reconstitute dried mouse monoclonal anti-human IgG4 antibody conjugated to colloidal gold incorporated therein. The mouse monoclonal anti-human IgG4 antibody conjugated to colloidal gold migrates from the reagent releasing end to the reaction zone and binds to the
antibody-antigen complex formed earlier thus forming a complex which comprises SXP/SXP-1 recombinant antigen, anti-filarial IgG4 antibodies and mouse monoclonal anti-human IgG4 antibody conjugated to colloidal gold. The presence of gold in the complex will result in the appearance of a red-purplish line at the location of the reaction zone indicating the presence of anti-filarial IgG4 antibodies in the sample tested. The unbound mouse monoclonal anti-human IgG4 antibody conjugated to colloidal gold from the reaction zone will further migrate to the control zone and bind with the anti-mouse IgG antibody, forming a red-purplish line in the control zone.

In a third embodiment of the present invention a biological sample such as blood, serum, plasma, urine or tears is first introduced to the sample receiving end of the absorbent nitrocellulose membrane and is allowed to migrate laterally via capillary action towards the reaction zone of the membrane. The anti-filarial IgG4 antibodies present in the sample will bind to the SXP/SXP-1 recombinant antigen immobilized within the reaction zone, forming an antibody-antigen complex or immunocomplex. A buffer is then introduced to the reagent releasing end to reconstitute dried mouse monoclonal anti-human IgG4 antibody conjugated to colloidal gold incorporated therein. The mouse monoclonal anti-human IgG4 antibody conjugated to colloidal gold migrates from the reagent releasing end to the control zone and binds with the anti-mouse IgG antibody, forming a red-purplish line in the control zone. The unbound mouse monoclonal anti-human IgG4
antibody conjugated to colloidal gold will further migrate to the reaction zone and binds to the antibody-antigen complex formed earlier thus forming a complex which comprises SXP/SXP-1 recombinant antigen, anti-filarial IgG4 antibodies and mouse monoclonal anti-human IgG4. antibody conjugated to colloidal gold. The presence of gold in the complex will result in the appearance of a red-purplish line at the location of the reaction zone indicating the presence of anti-filarial IgG4 antibodies in the sample tested.

The rapid immunochromatography assay was then evaluated using various categories of serum samples. The results of the evaluation as tabulated in Table 1 and 2 demonstrate the sensitivity and specificity of the immunochromatography assay of the present invention:

(a) Sensitivity:

<table>
<thead>
<tr>
<th>W. bancrofti serum sample</th>
<th>No.</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active infection</td>
<td>69</td>
<td>68</td>
<td>1</td>
</tr>
<tr>
<td>(microfilaria positive)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute infection, CFA+</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Chronic infection, CFA+</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>76</td>
<td>75</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 1 showing the sensitivity of the present method to detect *Wuchereria bancrofti* infection was 75/76 = 98.6%
(b) Specificity

<table>
<thead>
<tr>
<th>Source of serum sample</th>
<th>No</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy normals (from Malaysia)</td>
<td>308</td>
<td>0</td>
<td>308</td>
</tr>
<tr>
<td>Healthy normals from B. malayi endemic area</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>(tested negative by Brugia Rapid)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil-transmitted helminth infections</td>
<td>41</td>
<td>1</td>
<td>40</td>
</tr>
<tr>
<td>(Ascaris, Trichuris, Hookworm, Toxocariosis)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extraintestinal amoebiasis</td>
<td>64</td>
<td>0</td>
<td>64</td>
</tr>
<tr>
<td>Toxoplasmosis</td>
<td>15</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Taeniasis</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Malaria</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>TOTAL</td>
<td>438</td>
<td>1</td>
<td>437</td>
</tr>
</tbody>
</table>

Table 2 showing the specificity of the present method was 437/438 = 99.8%.

Further included in this invention is a diagnostic kit for rapid detection of lymphatic filariasis employing the methods described above. In one embodiment of the present invention, the kit comprises a detection device wherein the detection device contains an absorbent nitrocellulose membrane. The nitrocellulose membrane has three zones: a sample receiving end, a reaction zone and a control zone characterized by the SXP/SXP-1 recombinant antigen.
immobilized within the reaction zone. Anti-mouse IgG antibody is immobilized within the control zone. The detection device preferably is in a dipstick format. The kit further provides a microwell containing dried mouse monoclonal anti-human IgG4 antibody conjugated to colloidal gold. The kit also comprises of a buffer wherein the buffer comes in a separate container from the detection device.

In another embodiment of the kit which comprises a detection device wherein the detection device contains an absorbent nitrocellulose membrane. The nitrocellulose membrane has four zones; a sample receiving end, a reagent releasing end, a reaction zone and a control zone characterized by the SXP/SXP-1 recombinant antigen immobilized within the reaction zone. Mouse monoclonal anti-human IgG4 antibody conjugated to colloidal gold is incorporated within the reagent releasing end and anti-mouse IgG antibody is immobilized within the control zone of the membrane. The detection device may be in a dipstick or cassette format. The kit also comprises of a buffer wherein the buffer comes in a separate container from the detection device.

A volume of sample ranging from 10 to 50 μl is required for the rapid immunochromatography assay and result is obtained within 15 to 20 minutes. If a sample contains anti-filarial IgG4 antibodies specific to SXP/SXP-1 recombinant antigen, two red-purplish lines will be observed, each in the reaction zone and control zone. If the sample does not contain anti-
filarial IgG4 antibodies specific to SXP/SXP-1 recombinant antigen, the complex SXP/SXP-1 recombinant antigen, anti-filarial IgG4 antibodies and mouse monoclonal anti-human IgG4 antibody conjugated to colloidal gold will not be formed, thus no red-purplish line will be seen in the reaction zone, resulting in the final appearance of only one red-purplish line in the control zone. In conclusion two red-purplish lines on the nitrocellulose membrane denotes a positive test result and one line denotes a negative test result as shown in Figure 3.

While particular embodiments of the subject invention have been described, it will be obvious to those skilled in the art that various changes and modifications to the subject invention can be made without departing from the scope of the invention. It is intended to cover, in the appended claims, all such modifications that are within the scope of this invention.
What is claimed is:

1. A method for rapid detection of lymphatic filariasis in a biological sample, comprising the steps of:

   a) Adding a buffer to reconstitute a mouse monoclonal anti-human IgG4 antibody conjugated to a detection reagent prepared separately in a microwell;

   b) Adding a biological sample to the sample receiving end of a chromatographic element;

   c) Allowing the biological sample to flow from the sample receiving end to the reaction zone of the chromatographic element wherein SXP/SXP-1 recombinant antigen is immobilized within the reaction zone;

   d) Placing the chromatographic element in (c) into the microwell containing the reconstituted mouse monoclonal anti-human IgG4 antibody conjugated to a detection reagent in (a);

   e) Allowing the mouse monoclonal anti-human IgG4 antibody conjugated to a detection reagent to flow from the microwell to the reaction zone of the chromatographic element; and

   f) Detecting the complex formed in (e).

2. The method of claim 1 wherein the method further comprises the steps of:

   g) Allowing the unbound mouse monoclonal anti-human IgG4 antibody conjugated to a detection reagent in (e) to further flow from the reaction zone to the control zone of the chromatographic element wherein anti-mouse IgG antibody is immobilized within the control zone; and
3. A method for rapid detection of lymphatic filariasis in a biological sample, comprising the steps of:

a) Adding a biological sample to the sample receiving end of a chromatographic element;

b) Allowing the biological sample to flow from the sample receiving end to the reaction zone of the chromatographic element wherein SXP/SXP-1 recombinant antigen is immobilized within the reaction zone;

c) Adding a buffer to the reagent releasing end of the chromatographic element to reconstitute the dried mouse monoclonal anti-human IgG4 antibody conjugated to a detection reagent incorporated therein;

d) Allowing the mouse monoclonal anti-human IgG4 antibody conjugated to a detection reagent to flow from the reagent releasing end to the reaction zone of the chromatographic element; and

e) Detecting the complex formed in (d).

4. The method of claim 3 wherein the method further comprises the steps of:

f) Allowing the unbound mouse monoclonal anti-human IgG4 antibody conjugated to a detection reagent in (d) to further flow from the reaction zone to the control zone of the chromatographic element wherein anti-mouse IgG antibody is immobilized within the control zone; and

g) Detecting the complex formed in (f).
5. A method for rapid detection of lymphatic filariasis in a biological sample, comprising the steps of:

a) Adding a biological sample to the sample receiving end of a chromatographic element;

b) Allowing the biological sample to flow from the sample receiving end to the reaction zone of the chromatographic element wherein SXP/SXP-1 recombinant antigen is immobilized within the reaction zone;

c) Adding a buffer to the reagent receiving end of the chromatographic element to reconstitute the dried mouse monoclonal anti-human IgG4 antibody conjugated to a detection reagent incorporated therein;

d) Allowing the mouse monoclonal anti-human IgG4 antibody conjugated to a detection reagent to flow from the reagent releasing end to the control zone of the chromatographic element wherein anti-mouse IgG antibody is immobilized within the control zone;

e) Allowing the mouse monoclonal anti-human IgG4 antibody conjugated to a detection reagent to further flow from the control zone to the reaction zone of the chromatographic element; and

f) Detecting the complex formed in (d) and (e).

6. The method according to any one of claims 1 to 5 wherein the method is for detection of *Wuchereria bancrofti* and/or *Brugia malayi* and/or *B* *rugia *t* *imbori* infections.

7. The method according to any one of claims 1 to 6 wherein the chromatographic element is a membrane or any other suitable chromatographic element.
8. The method of claim 7 wherein the membrane is an absorbent nitrocellulose membrane.

9. The method of claim 7 wherein the other suitable chromatographic elements include nylon and/or mixed esters.

10. The method according to any one of claims 1 to 9 wherein the recombinant antigen is expressed by SXP/SXP-I gene in Brugia malayi and/or IVuchereria bancrofti and/or Brugia timori.

11. The method according to any one of claims 1 to 10 wherein the detection reagent is gold particles, latex particles or any other suitable particles.

12. The method of claim 11 wherein the other suitable particles include any one of silver or non-metal colloidal particles such as selenium, telluriura and sulfur.

13. A diagnostic kit for rapid detection of lymphatic filariasis in a biological sample according to the method of any one of claims 1 to 12 comprising a detection device wherein the detection device comprises a chromatographic element wherein the chromatographic element comprises a sample receiving end, a reaction zone and a control zone characterized in that SXP/SXP-I recombinant antigen is immobilized within the reaction zone and dried mouse monoclonal anti-human IgG4 antibody conjugated to a detection reagent is in a separate rhicrowell.
14. A diagnostic kit for rapid detection of lymphatic filariasis in a biological sample according to the method of any one of claims 1 to 12 comprising a detection device wherein the detection device comprises a chromatographic element wherein the chromatographic element comprises a sample receiving end, a reagent releasing end, a reaction zone and a control zone characterized in that SXP/SXP-1 recombinant antigen is immobilized within the reaction zone.

15. The diagnostic kit of claim 13 or 14 wherein the kit further comprises a buffer wherein the buffer is in a separate container from the detection device.

16. The diagnostic kit of claim 14 wherein mouse monoclonal anti-human IgG4 antibody conjugated to a detection reagent is immobilized within the reagent releasing end of the chromatographic element.

17. The diagnostic kit according to any one of claims 13 to 16 wherein the detection reagent is gold particles, latex particles or any other suitable particles.

18. The diagnostic kit of claim 17 wherein the other suitable particles include any one of silver or non-metal colloidal particles such as selenium, tellurium and sulfur.
19. The diagnostic kit according to any one of claims 13 to 18 wherein anti-mouse IgG antibody is immobilized within the control zone.

20. The diagnostic kit according to any one of claims 13 to 19 wherein the chromatographic element is a membrane or any other suitable chromatographic element.

21. The diagnostic kit of claim 20 wherein the membrane is an absorbent nitrocellulose membrane.

22. The diagnostic kit of claim 20 wherein the other suitable chromatographic elements include nylon and/or mixed esters.

23. The diagnostic kit according to any one of claims 13 to 22 wherein the kit is for detection of Wuchereria bancrofti and/or Brugia malayi and/or Brugia timori infections.

24. The diagnostic kit according to any one of claims 13 to 23 wherein the recombinant antigen is expressed by SXP/SXP-1 gene in Brugia malayi and/or Wuchereria bancrofti and/or Brugia timori.

25. The diagnostic kit according to any one of claims 13 to 24 wherein the detection device is a dipstick.

26. The diagnostic kit according to any one of claims 13 to 24 wherein the detection device is a cassette.
27. The use of a diagnostic kit according to any one of claims 13 to 26 for the detection of lymphatic filariasis in a biological sample.
Figure 1
Figure 2.
A. CLASSIFICATION OF SUBJECT MATTER

GOI N 33/53 (2006.01) i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKIPASS (KIPO Internal), USPTO, Espacenet, Delphion, Google Scholar ("lymphatic filariasis, SXP, immunochromatography etc")

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No</th>
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<tbody>
<tr>
<td>Y</td>
<td>P J Lammie et al., &quot;Recombinant antigen-based antibody assays for the diagnosis and surveillance of lymphatic filariasis-a multicenter trial&quot; in Filat J 2004, Vol 3 9; See Abstract and Materials &amp; Methods</td>
<td>1-6</td>
</tr>
<tr>
<td>Y</td>
<td>P Lahtha et al., &quot;Development of antigen detection ELISA for the diagnosis of Brugian and Bancrofian Filatiasis using antibodies to recombinant filarial antigens Bm-SXP-I and WB-SXP-I&quot; in Microbiol Immunol 2002 Vol 46(5) pages 327-332; See Materials and Methods</td>
<td>1-6</td>
</tr>
<tr>
<td>Y</td>
<td>L K V Baskar et al., &quot;Development and evaluation of a rapid flow through immuno filtration test using recombinant filarial antigen for diagnosis of brugian and bancrofian filariasis&quot; in Microbiol Immunol 2004 Vol 48(7) pages 519-525; See Materials and Methods</td>
<td>1-6</td>
</tr>
</tbody>
</table>

[X] Further documents are listed in the continuation of Box C

☐ See patent family annex

* Special categories of cited documents

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search: 22 AUGUST 2007 (22 08 2007)

Date of mailing of the international search report: 22 AUGUST 2007 (22.08.2007)

Name and mailing address of the ISA/KR

Korean Intellectual Property Office

920 Dunsan-dong, Seo-gu, Daejeon 302-701, Republic of Korea

Authorized officer

CHO, Myung Sun

Telephone No 82-42-481-5594

Facsimile No 82-42-472-7140

Form PCT/ISA/210 (second sheet) (April 2007)
**INTERNATIONAL SEARCH REPORT**

**Box No. II**  Observations  where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos because they relate to subject matter not required to be searched by this Authority, namely

2. [X] Claims Nos 8,9,12,16,18,21,22 because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically

   They refer dependent claims which are not drafted in accordance with the second and third sentence of Rule 6-4(a), hindering a meaningful search

3. [X] Claims Nos 7,10,11,13-15,17,19,20,23-27 because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6-4(a)

**Box No. III**  Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims, it is covered by claims Nos

**Remark on Protest**

[X] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee

[X] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation

[X] No protest accompanied the payment of additional search fees

Form PCT/ISA/210 (continuation of first sheet (2)) (April 2007)
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>N. Rahmah et al., &quot;Comparison of IgG4 assays using whole parasite extract and BmRl recombinant antigen in determining antibody prevalence in brugian filariasis&quot; in Filaπa J 2004 Vol 3 8 See entire document</td>
<td>1-6</td>
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