Realizing the Full Cycle of Research and Development: Lymphatic Filariasis

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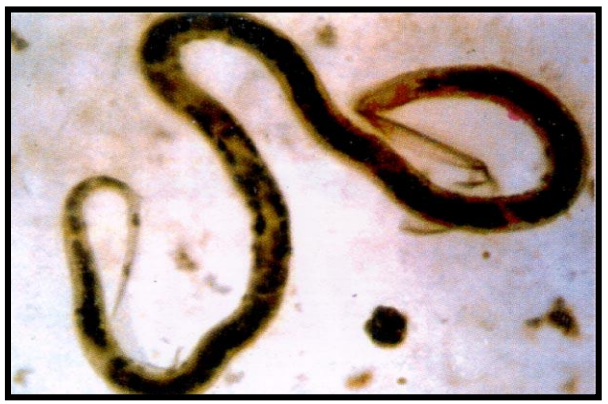
THE DISEASE

Lymphatic Filariasis (LF) is a parasitic infectious disease caused by three main species of blood/tissue worms:

***Wuchereria bancrofti*, *Burgia malayi* and *Brugia timori*.**

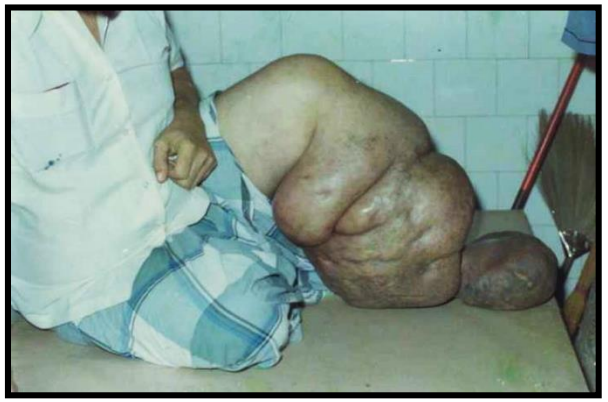
The disease is transmitted by several species of mosquitoes. Infective larvae from mosquitoes enter the human body after the mosquito bite. The larvae then travel to lymphatic channels and lymph nodes and develop into adult worms.



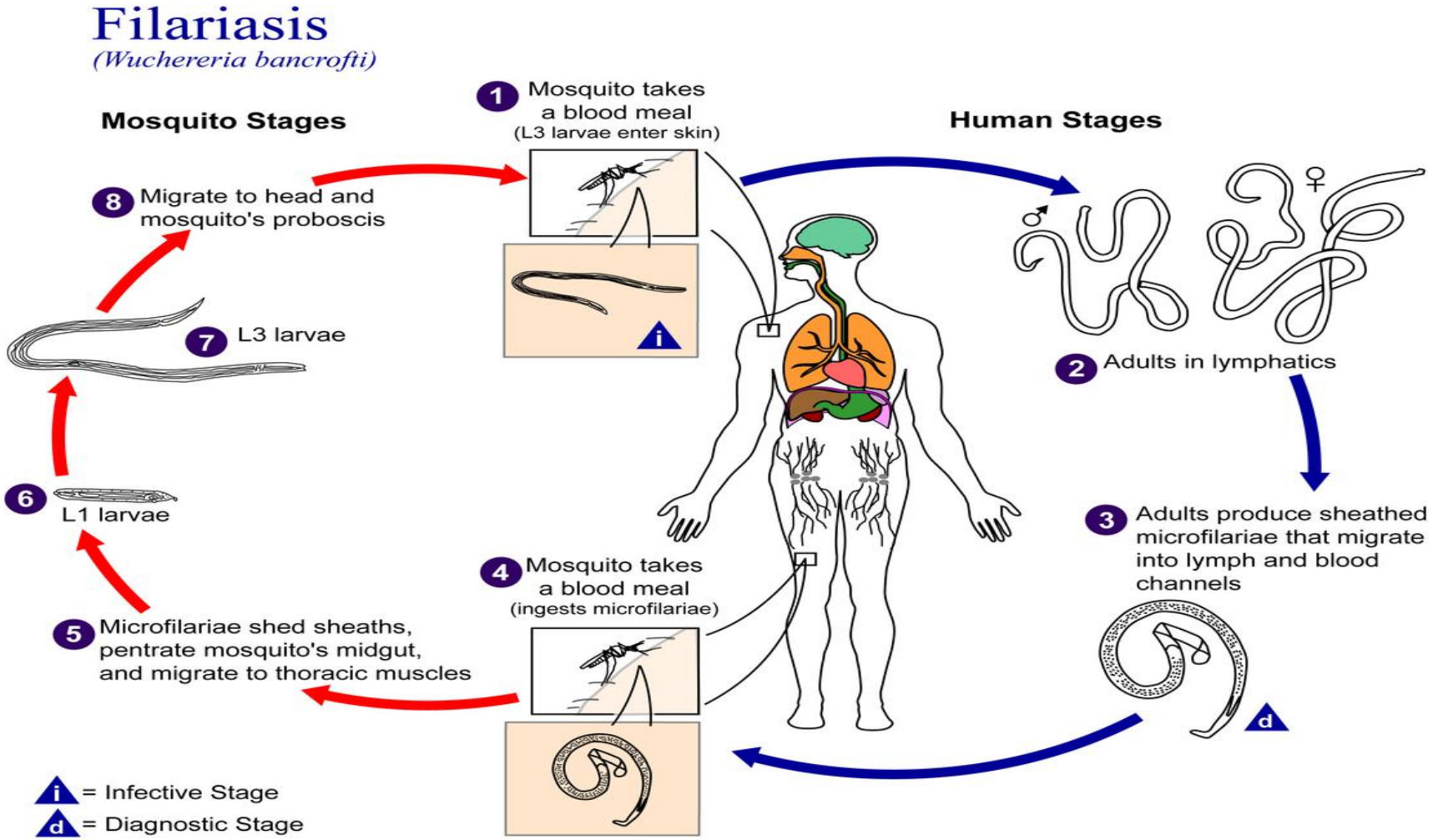
THE DISEASE

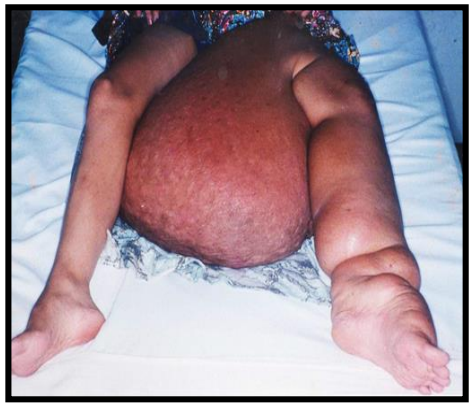
Brugia malayi **is endemic in several Asian countries including India, China, Indonesia, Thailand, Vietnam, Philippines and Malaysia. About 13 million people are infected and this figure are at risk of acquiring brugian filariasis.**

Infection with *Brugia malayi* produce one or more of the following symptoms and manifestations: recurrent fever, lymphatic & renal damage, adenolymphangitis, elephantiasis and pulmonary disease.



THE DISEASE

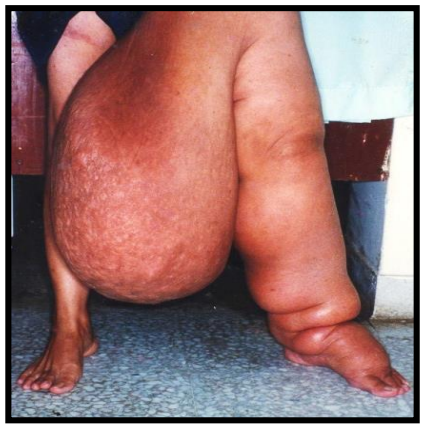

Filariasis
(Wuchereria bancrofti)
Mosquito Stages
Human Stages
1 Mosquito takes a blood meal (L3 larvae enter skin)
2 Adults in lymphatics
3 Adults produce sheathed microfilariae that migrate into lymph and blood channels
4 Mosquito takes a blood meal (ingests microfilariae)
5 Microfilariae shed sheaths, pentrate mosquito's midgut, and migrate to thoracic muscles
6 L1 larvae
7 L3 larvae
8 Migrate to head and mosquito's proboscis
i = Infective Stage
d = Diagnostic Stage



INTRODUCTION

The recent influx of foreign workers from neighboring countries which are endemic with LF are causing concern about the possibility of a rise in LF in Malaysia in the near future.

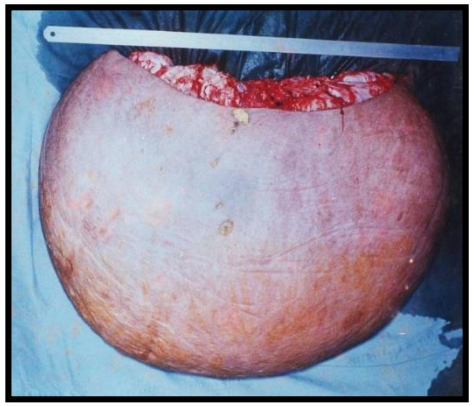
The growth of LF elimination program has highlighted the need for tools that can be used to monitor progress and conduct surveillance to detect any potential resumption of transmission.



Medicine & International Health, 1998. 3(3): p. 184-188

INTRODUCTION

The reference test, microscopy detection of microfilariae, is insensitive and thus newer diagnostic methods have been developed such as the polymerase chain reaction (PCR) based assays and detection of anti-filarial IgG4 antibody.



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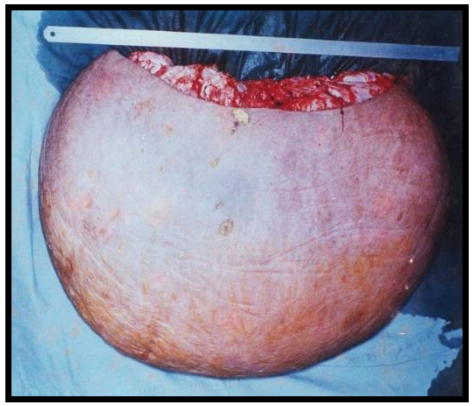
PROBLEM STATEMENT

The lack of a highly specific and sensitive serology-based diagnostic test for Brugia infections that can overcome the challenging task of nocturnal blood collection is required.

INTRODUCTION

Development of an antigen detection method such as that available for bancroftian filariasis (More and Copeman'1990) or a specific & sensitive antibody detection test would allow:

1. **Ease of performance,**
2. **Day blood sampling,**
3. **Cost reduction.**



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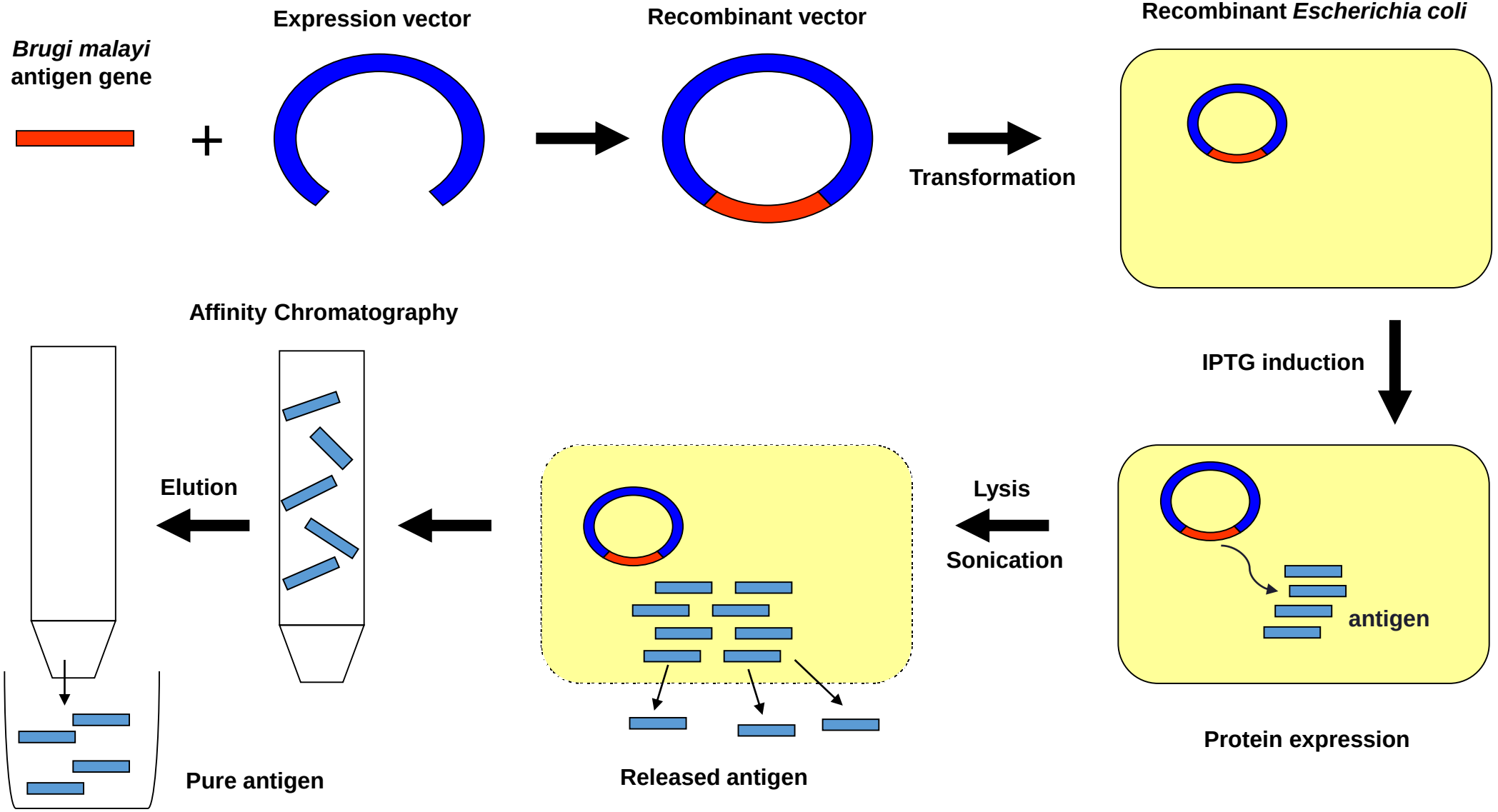
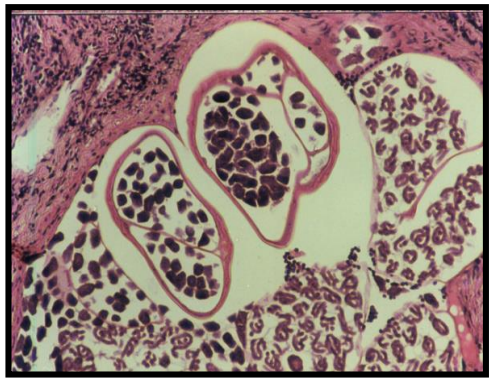
Recombinant antigen (BmR1)



Brugia-ELISA

Brugia malayi **recombinant antigen (BmR1) that demonstrate specific reaction with Brugian filariasis sera in Western blots. The recombinant antigen was subsequently optimized to be used in an indirect Enzyme Linked Immunosorbent Assay (Brugia ELISA)**

A study was conducted to evaluate, Brugia-ELISA using 2487 serum samples to determine its usefulness in the detection of ***Brugia malayi*** **infection.**



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Recombinant antigen (BmR1)

100µl of diluted antigen coated in each of 96-well plate

↓ incubated overnight in 4-6°C / washed 3X with PBS-Tween, tapped dry

100 µl of test sera added to each well

↓ incubated for 2 hours at room temperature / washed 3X with PBS-Tween, tapped dry

100 µl of diluted anti human IgG4-HRP

↓ incubated for 30 min at room temperature / washed 3X with PBS-Tween, tapped

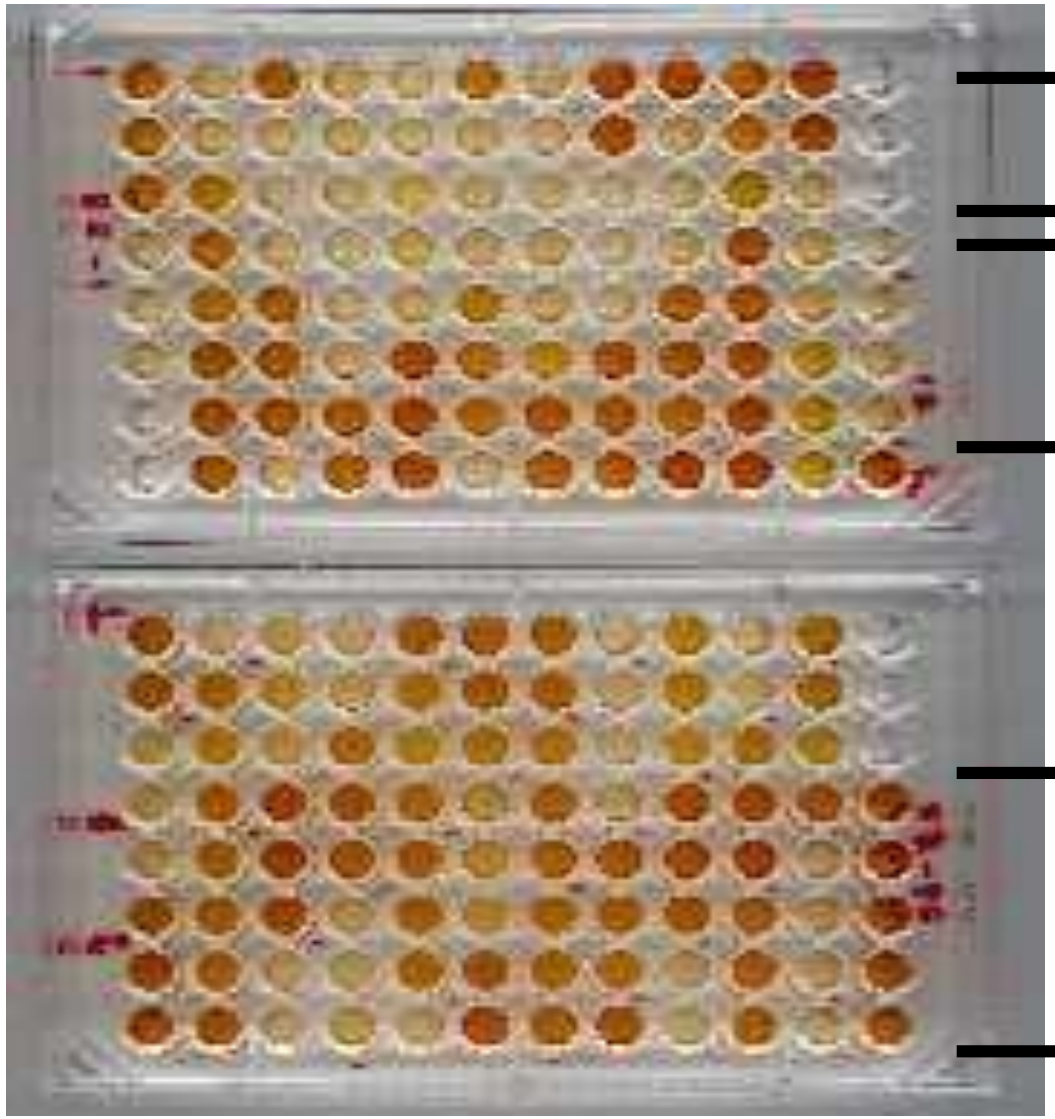
100 µl of ABTS substrate

↓ incubated for 30 min. in the dark, at room temperature

50 µl of H_2SO_4

read plate using ELISA reader at OD 410 nm

ELISA against BmR1



Blank PBS

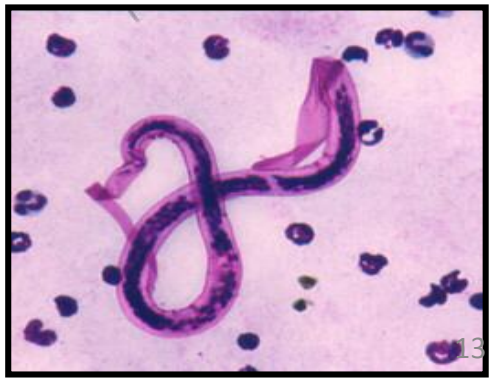
Negative samples

Positive samples

Brugia-ELISA

Anti-filarial IgG4-ELISA is useful in detecting active *B. malayi* infection provided that an appropriate cut-off value is defined. The assay can also be used in prevalence determination as replacement to mf detection or as an epidemiological tool.

As finger prick blood is suitable for the detection of specific IgG4 by ELISA without compromising sensitivity of the test, **anti-filarial IgG4 levels** can now be **easily measured in the community**.



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RESULTS

Evaluation of Brugia-ELISA using sera from UNIVERSITI SAINS MALAYSIA (USM)

Grp	Serum samples	n	Positive	Negative
1	*Brugia malayi* microfilaraemics	80	80(100%)	0
2	chronic cases (mf-, early and late chronic stages)	24	5(20.8%)	19(79.2%)
3	Mf-, endemic area individuals with high titres of anti-filarial IgG4 to CSA	85	85(100%)	0
4	Mf-, endemic area individuals with low titres of anti-filarial IgG4 to CSA	527	0	527(100%)
5	Mf-, endemic area individuals with positive PCR-ELISA results	16	16(100%)	0
6	Mf-, endemic area indivuals with negative PCR-ELISA results	165	0	165(100%)
7	*Brugia timori* microfilaraemics	2	2(100%)	0
8	*W.bancrofti* microfilaraemics	22	12(54.5%)	10(45.5%)
9	*W.bancrofti* chronic cases (mf-)	10	1(10%)	9(90%)
10	Normal endemics from bancroftian region	20	0	20(100%)

RESULTS

Evaluation of Brugia-ELISA using sera from Universiti Sains Malaysia (USM)

Grp	Serum samples	n	Positive	Negative
11	Soil-transmitted helminthiases (*Ascaris, Trichuris,* hookworm)	100	0	100(100%)
12	Other helminthic diseases (*D.immitis, O.volvulus, L.loa, G.spinegerum, C.sinensis, Toxocara, A.simplex, S.stercoralis, S.mansoni*)	24	0	24(100%)
13	Protozoal infections (*P.falciparum, T.gondii, E.histolytica*)	46	0	46(100%)
14	Bacterial infections (*S.typhi, M.tuberculosis, C.jejuni, E.coli* O157, *S.dysenteriae*)	10	0	10(100%)
15	Healthy individuals from non-endemic areas	900	0	900(100%)
	Total	**2031**		

RESULTS

Evaluation of Brugia-ELISA using sera from INSTITUTE OF MEDICAL RESEARCH (IMR)

Grp	Serum samples	*n*	Positive	Negative
1	Microfilaraemic brugian filariasis sera	25	24(96%)	1(4%)
2	Non-endemic sera from patients with other parasitic infections (toxoplasmosis, amoebiasis, malaria)	92	4(4.3%)	88(95.7%)
3	Clinically suspected chronic elephantiasis patients with indirect immunofluorescence assay positive for filariasis	13	1(7.7%)	12(92.3%)
4	Amicrofilaraemic endemic `normals'	20	6(30%)	14(70%)
	Total	**150**		

RESULTS

Evaluation of Brugia-ELISA using sera from UNIVERSITI OF MALAYA (UM)

Grp	Serum samples	*n*	Positive	Negative
1	Microfilaraemic brugian filariasis sera	6	6(100%)	0
2	Endemic area with strong clinical diagnosis of early chronic filariasis	3	3(100%)	0
3	High IgM titre for *T.gondii* infection	108	3(2.8%)	105(90.2%)
4	Other parasitic infections: (amoebiasis, toxocariasis, schistosomiasis, gnathostorniasis, leshmaniasis & malaria)	9	0	9(100%)
	Total	**126**		

RESULTS

Evaluation of Brugia-ELISA using sera from INDIA

Grp	Serum samples	*n*	Positive	Negative
1	Microfilaria positive	30	30(100%)	0
2	Patients treated 1 year ago with DEC	30	21(70%)	9(30%)
3	Early chronic patients	15	9(60%)	6(40%)
4	Late chronic patients	15	13(87%)	2(13%)
5	Endemic normals (amicrofilaraemics from endemic areas with no apparent clinical symptoms)	30	3(10%)	27(90%)
6	Non-endemic normals	20	0	20(100%)
	Total	**140**		

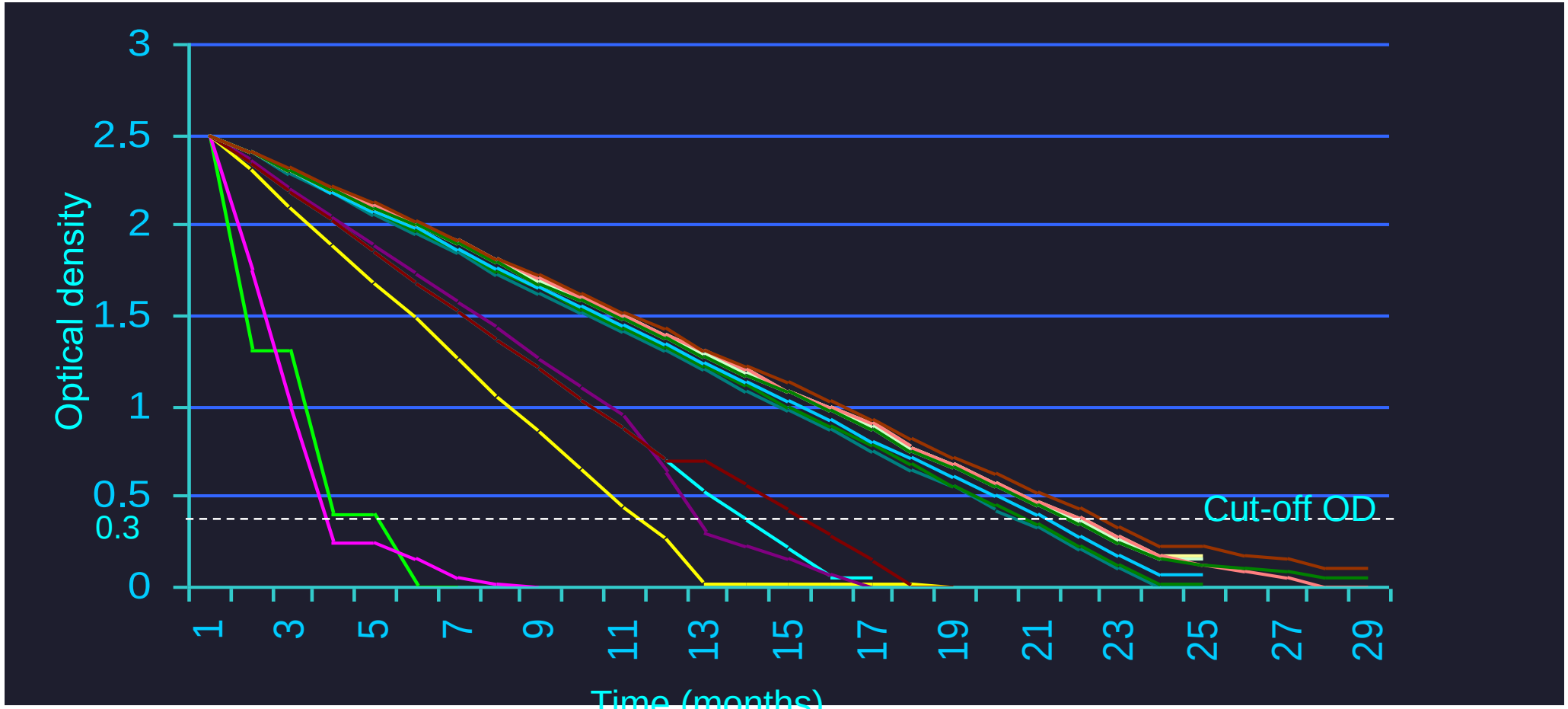
RESULTS

Evaluation of Brugia-ELISA using sera from THAILAND

Grp	Serum samples	*n*	Positive	Negative
1	Microfilaria positive	4	4(100%)	0
2	Completely treated individuals. Treatment regimen in Thailand is as follows: 1 DEC course (300 mg/day for 6 days) repeated every 6 months for 2 years	15	0	15(100%)
3	Endemic area residents with clinical symptoms of early chronic stage	6	0	6(100%)
4	Endemic normals	6	6(100%)	0
5	Non-endemic areas, with soil-transmitted helminthiasis (hookworm, *Ascaris lumbricoides, Strongyloides stercoralis*).	9	0	9(100%)
	Total	**40**		

RESULTS

Follow-up by Brugia-ELISA of 15 brugian filariasis patients post-chemotherapy with a 6-day course of diethylcarbamazine (6mg/kg bodyweight)



RESULTS

Sensitivity and specificity of Brugia-ELISA for the diagnosis of brugian filariasis as assessed by serum samples from four institutions.

Institution	*n*	Specificity	Sensitivity	PPV	NPV
IMR	117	95.6%	96.0%	85.7%	98.9%
UM	126	97.4%	100%	75.0%	100%
India	50	100%	100%	100%	100%
Thailand	40	100%	100%	100%	100%
Total	333				

IMR, Institute of Medical Research (Malaysia); UM, University of Malaya; PPV, positive predictive value; NPV, negative predictive value

DISCUSSION

The routine laboratory diagnosis for *B. malayi* infection still relies on the microscopy confirmation of microfilariae by examination of stained thick blood smears and/or blood concentration using Knott's method or membrane filtration.

Although these methods are cheap and specific, they require night-blood samples and are known to be insensitive; therefore these techniques will not detect low microfilaraemia levels, single-sex infections and amicrofilaraemic stages of the infection (TURNER et al., 1992).

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DISCUSSION

In the global programme for elimination of bancroftian filariasis, antigen-detection assays are available as diagnostic tools. However, no equivalent antigen test exist for brugian filariasis despite the many attempts aimed at its development.

Thus for Brugia infections, there is still a great need for a very specific and sensitive serology-based diagnostic method. Various investigators have shown that IgG4 antibodies to lymphatic filarial parasites indicate active infection (RAHMAH et al.,1998b); thus assays based on this antibody isotype would be promising as immunodiagnostic test (HAARBRINK et al., 1999).

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DISCUSSION

Although PCR-based assays can also be employed to detect Brugia infection, they are not suitable for large-scale screening. The availability of a recombinant antigen that is specific and sensitive for diagnosis of *Brugia malayi* infection will allow for an almost unlimited supply of a well-characterized antigen as compared to worm-derived antigen, thus making it suitable for mass production as a diagnostic test.

Previous reports of recombinant antigen for diagnosis of brugian filariasis have not shown high specificity and high sensitivity (CHANRASHEKAR et al., 1994; DISSANAYAKE et al., 1994).

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DISCUSSION

We have produced a novel recombinant antigen that reacted specifically with sera of *Brugia malayi*-infected patients and subsequently an enzyme-linked immunosorbent assay (ELISA)-based method named (Brugia-ELISA) was developed.

In this study; we report the results of evaluations of this test using a total of 2487 sera from 5 institutions. Except for majority of USM sera, all other samples were blinded, i.e., the identity of the samples were known only after the ELISA result were obtained.

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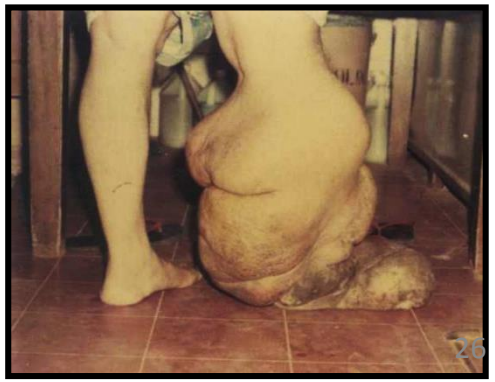
DISCUSSION

The evaluations covered a wide range of sera, thus providing a fairly comprehensive picture of the usefulness of this test.

The findings that sera from patients with various helminthic infections (other than *Wuchereria bancrofti*) did not cross-react with this recombinant antigen are significant, since these sera are often responsible for cross-reactivities and hence the low specificity seen in serological tests for filariasis.



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DISCUSSION

These evaluation studies demonstrated the high specificity and high sensitivity of Brugia-ELISA in detecting *B. malayi* infection.

This assay would be a very useful tool in patient diagnosis, in monitoring success of treatment, and in the filariasis elimination programmes.

We have now developed an immunochromatography-based dipstick format of this test named ***Brugia RAPID*** which is advantageous since it can be stored at room temperature, is user-friendly, rapid and field-applicable.

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CERTIFICATE OF A PATENT

MALAYSIA

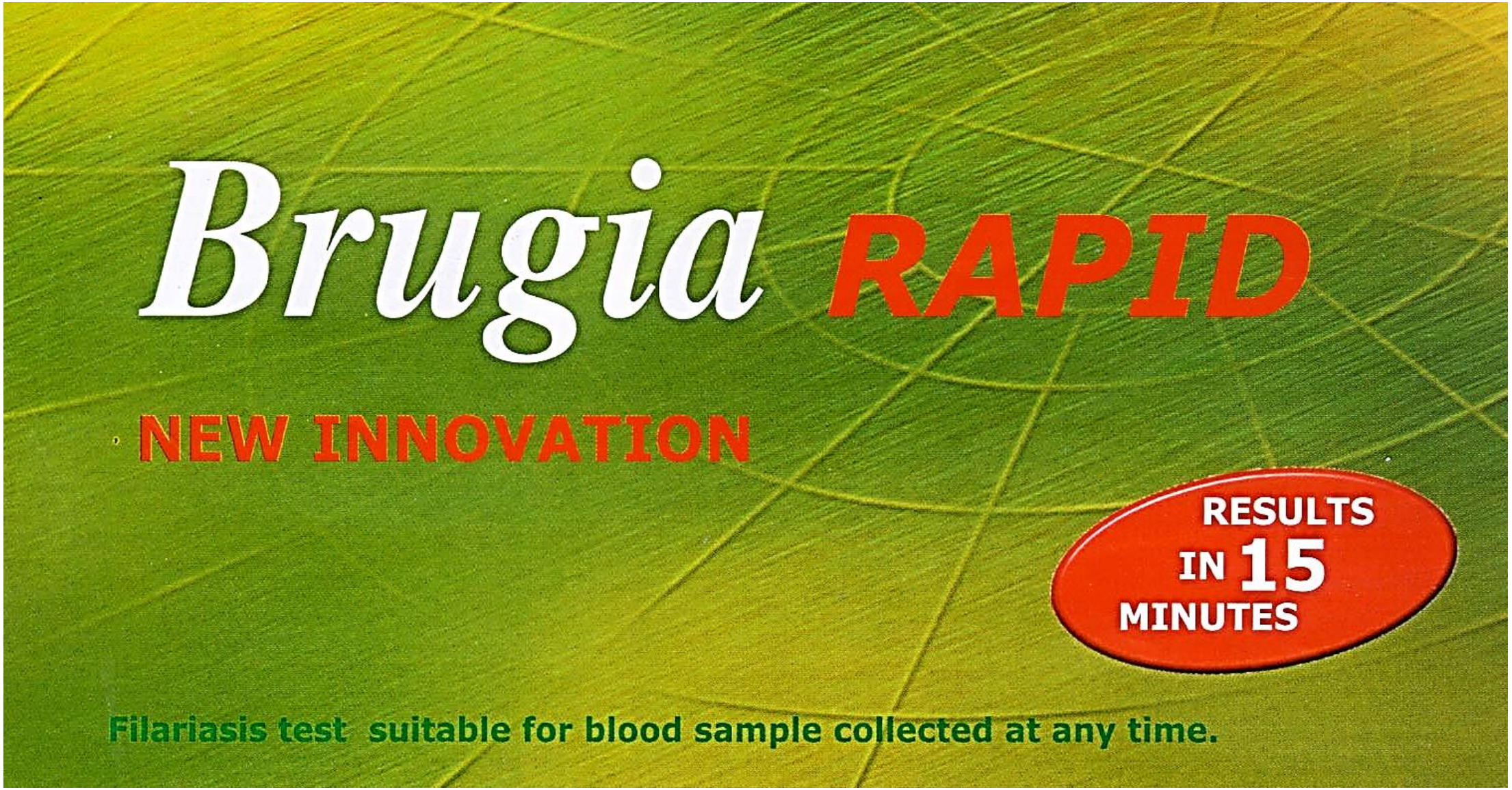
CERTIFICATE OF GRANT OF A PATENT

In accordance with Section 31(2) of the Patents Act 1983 a patent for an invention having grant number MY - 134618 - A has been granted to UNIVERSITI SAINS MALAYSIA in respect of an invention having the following particulars :

TITLE	:	FILARIAL PARASITE POLYPEPTIDES AND SEQUENCES, GENE SEQUENCES AND USES' THEREOF.
FILING DATE	:	24 DECEMBER 1999
PRIORITY DATE	:	NONE
NAME OF INVENTOR	:	DR. RAHMAH NOORDIN; PROFESOR (DR.) KHAIRUL ANUAR ABDULLAH.
PATENT OWNER	: :	UNIVERSITI SAINS MALAYSIA 11800 USM PULAU PINANG MALAYSIA.
DATE OF GRANT	:	31 DECEMBER 2007

Dated this 31 day of DECEMBER 2007

(SHAMSIAH BT. KAMARUDDIN)
for Registrar of Patents
MALAYSIA


Brugia RAPID
NEW INNOVATION
RESULTS IN 15 MINUTES
Filariasis test suitable for blood sample collected at any time.

Brugia RAPID

The routine approach for filariasis is the collection of night blood and the detection of the parasite larvae (microfilariae) by microscopy in the blood sample.

Brugia RAPID **represents an innovative, rapid and specific approach to diagnosis.**

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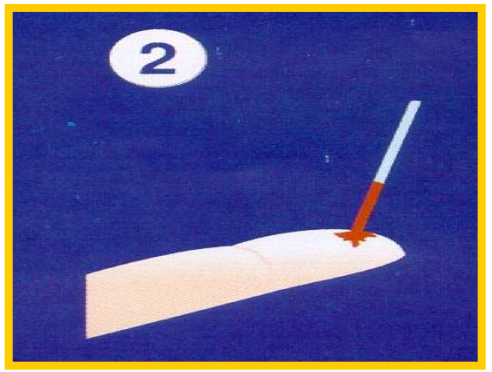
Brugia RAPID

1. The only rapid diagnostic assay available for Brugian filariasis;
2. Sample collected anytime of the day can be used;
3. Blood, serum or plasma can be used as a sample;
4. High sensitivity & specificity;
5. No refrigeration required;
6. Rapid test – 15 minutes;
7. Simple & convenient.

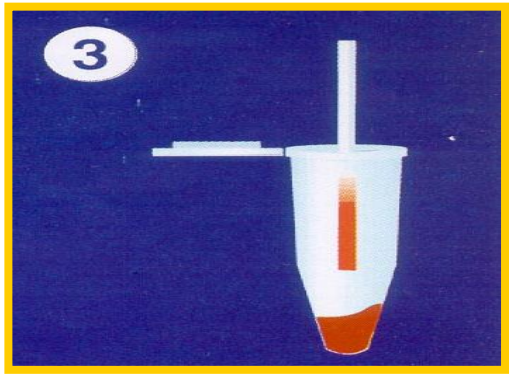
University Malaya Medical Centre

Brugia RAPID dipstick

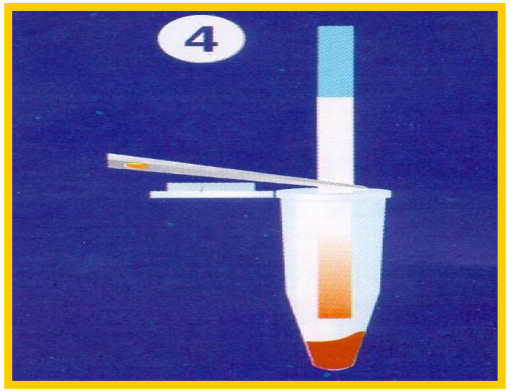
Place one drop of buffer into each tube and well



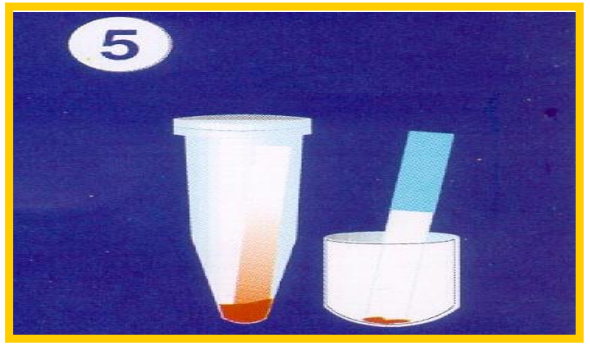
Obtain a capillary full of blood



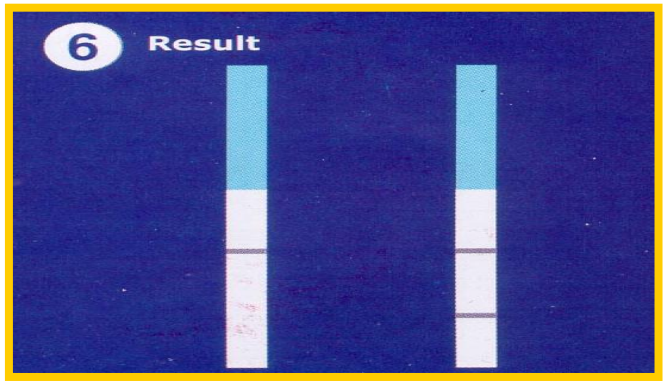
Place capillary tube into microfuge tube



When the sample front almost reaches the top pad, cut off blood filter



Place dipstick into the conjugate well. Leave for 10-15 min.



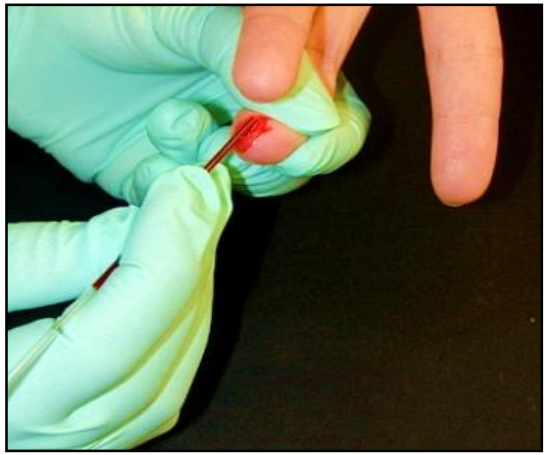
Negative Positive

Brugia RAPID cassette

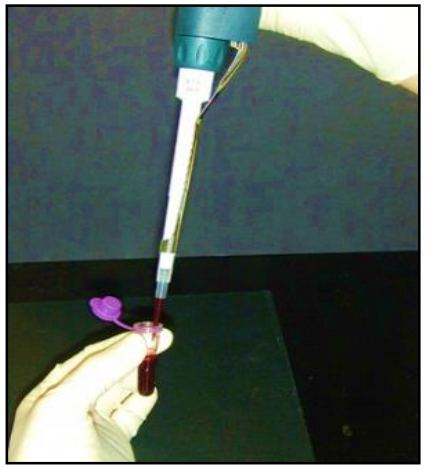
1

Bring test cassette and chase buffer to room temperature. Remove cassette from foil pouch just before use. Label the cassette with information on the sample.

2

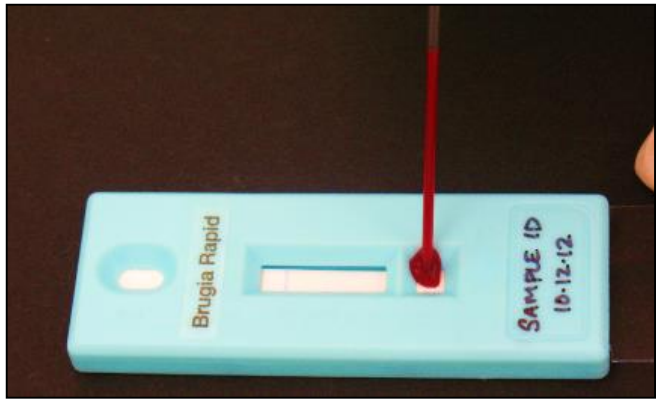


Collect 35 µl of blood by finger prick into a calibrated capillary tube *or* remove 35 µl of blood from a microcentrifuge tube with a micropipette. *Do not* add blood directly from the finger to the cassette.

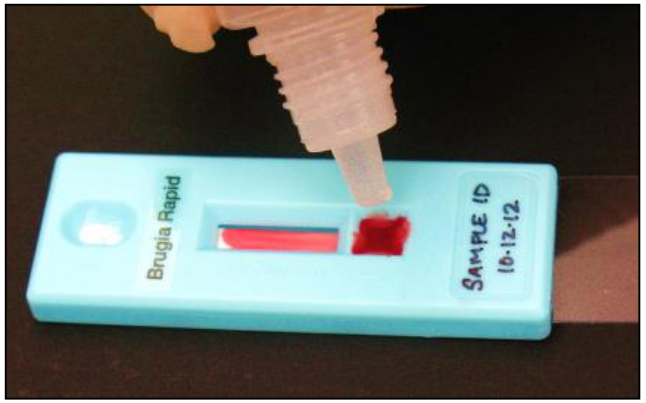


Brugia RAPID cassette

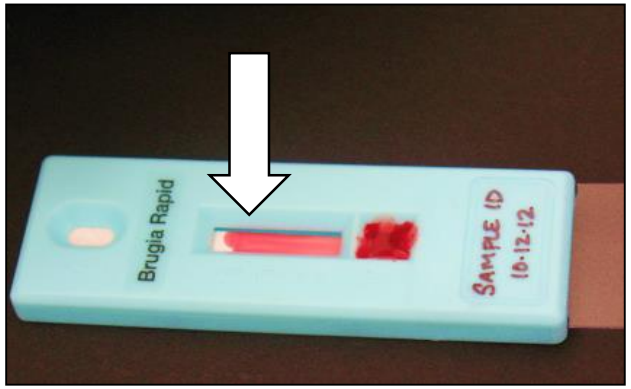
3



Add blood sample slowly to the square well by touching the capillary tube or pipette tip to the sloping side.



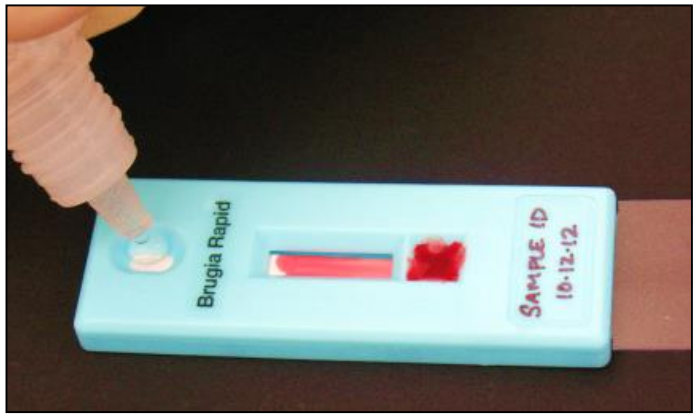
Add one drop of chase buffer to the same square well.



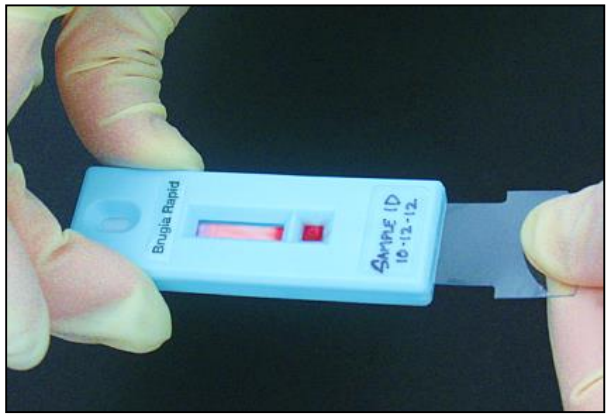
The sample will start to flow up the strip. The cassette can be tapped gently on the table to facilitate the flow. Wait until the sample has reached the blue line (A).

Brugia RAPID cassette

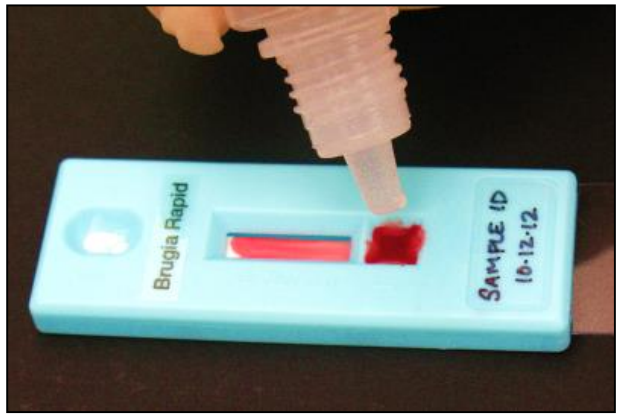
4



When the sample has reached the blue line (A), add three drops of chase buffer to the circle well at the top of the cassette.



Firmly pull the clear tab at the bottom of the cassette until you feel resistance.

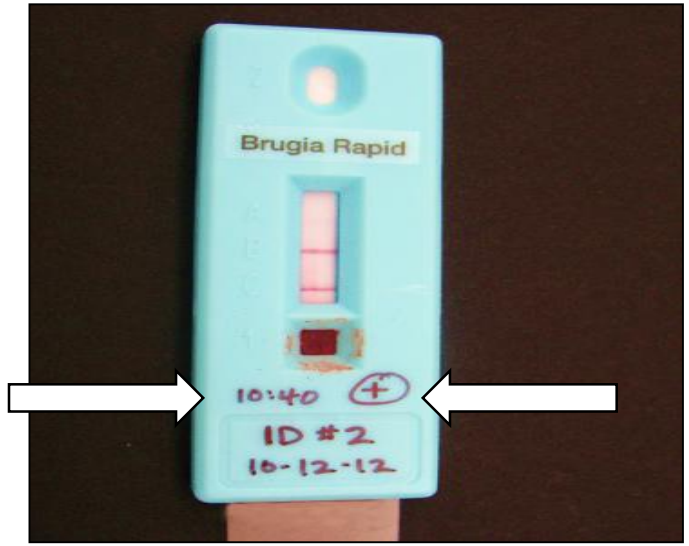


After pulling the clear tab, add one drop of buffer to the square well.

Brugia RAPID cassette

5

Start timing. Read test results 25 minutes after adding the final drop of buffer.



Record the start or end time on the front of the cassette.

RESULTS

Categories of sera from various institution used in the evaluation of *Brugia RAPID*

	Source of serum samples						
Category	USM	FKUI	TDMC	UM	IMR	UKM	Total
Microfilaria-+ve	39	108	30	10	20	0	207
STH	39	19	0	0	0	12	70
Other helminth	32	10	0	26	0	0	68
Protozoan infection	72	13	0	47	80	26	238
Other infection	12	0	0	0	0	0	12
Healthy person	101	8	20	17	0	12	158
Total	295	158	50	100	100	50	753

Except for the microfilaria-+ve serum samples, all other samples were from individuals residing in non-filaria-endemic areas. USM, Universiti Sains Malaysia; FKUI, University of Indonesia; TDMC, T.D. Medical College, India; UM, University Malaya, Malaysia; IMR, Institute for Medical Research, Malaysia; UKM, University Kebangsaan, Malaysia

RESULTS

Details of sera from other infections used in the evaluation of *Brugia RAPID*

Category and species	*n*	Category and species	*n*
STH:	**70**	**Protozoan infections:**	**238**
Ascaris lumbricoides	16	*Toxoplasma gondii*	118
Trichuris trichiura	21	*P. vivax / P. falciparum*	41
Hookworm	14	*Entamoeba histolytica*	72
Strongyloides stercoralis	1	*Iodamoeba butschlii*	2
Mixed infections	18	*Giardia lamblia*	2
Other helminth infections:	**68**	*Cryptosporidium parvuum*	2
Toxocara	31	*Leishmania*	1
Gnathostoma spinegerum	1	**Other infections:**	**12**
Taenia solium (cystercercosis)	11	Dengue	1
Dirofilaria immitis	5	Scrub typhus	1
Anasakis	8	Hepatitis	4
Schistosoma mansoni	9	*Salmonella typhi*	5
Onchocerca volvulus	3	*Campylobacter jejuni*	1
	TOTAL 388		

RESULTS

Evaluation results of *Brugia RAPID* dipstick test using sera from 6 institutions

Category	n	Brugia Rapid positive[a] (%)
Microfilaria +ve	207	200 (96.6%)
STH	70	1 (1.4%)
Other helminth infections	68	1 (1.5%)
Protozoal infections	238	2 (0.8%)
Other infections	12	0 (0%)
Healthy individuals	158	2 (1.3%)
Total	753	

[a]All other samples were *Brugia RAPID* negative

RESULTS

Specificity and sensitivity of *Brugia RAPID* dipstick test for the diagnosis of brugian filariasis, as assessed by serum samples from six institutions

	True positive (mf + ve)	True negative (mf -ve)	Total
Brugia Rapid + ve	200	6	206
Brugia Rapid - ve	7	540	547
Total	207	546	753

mf, microfilaria

Sensitivity: 200/207 = 96.6%

Specificity: 540/546 = 98.9%

Positive predictive value: 200/206 = 97.1%

Negative predictive value: 540/547 = 98.7%

RESULTS

Detection of *B. timori* microfilariae & IgG4 antibodies using *Brugia RAPID* dipstick test in residents with different signs of LF living in an endemic village, Indonesia

Clinical status [a]	Membrane filtration		Brugia Rapid	
	(mf +ve)%	(mf -ve)%	(mf +ve)%	(mf -ve)%
Lymphoedema (grade 1–3)	8 (8.2)	90 (91.8)	64 (65.3)	34 (34.7)
Adenolymphangitis/scars	31 (26.5)	86 (73.5)	92 (78.6)	25 (21.4)
Asymptomatic	157 (28.9)	387 (71.1)	455 (83.6)	89 (16.4)
Total	196 (25.8)	563(74.2)	611 (80.5)	148 (19.5)

[a] By physical and parasitological examination and questionnaire.

The study showed that the BR test designed for *B. malayi* was able to detect anti-filarial IgG4 antibodies in individuals living in an area highly endemic for B. timori. The BR test is thus a sensitive tool for assessment of specific IgG4 antibodies not only against B. malayi but also against B. timori.

RESULTS

Prevalence rates with 95% confidence intervals for anti-filarial antibodies based on *Brugia RAPID* dipstick test in children aged between 3 and 10 years of age prior to mass drug administration (2001) and after one or more rounds of MDA

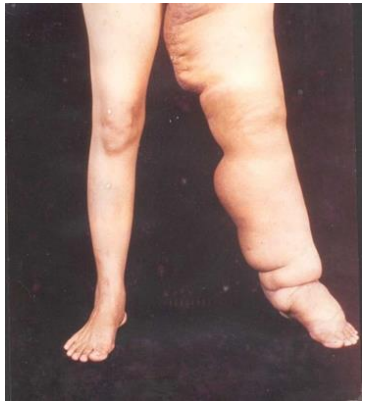
Year	N examined	% Brugia Rapid Positive	95% C
2001	120	82.0	75.1–88.8
2003	243	39.1	32.9–45.2
2004	202	41.1	34.3–47.9
2005	164	22.6	16.2–29.0
2006	138	13.0	7.4–18.6
2007	119	6.7	2.2–11.2
2008	115	13.9	7.4–20.2
2009	103	3.9	0.2–7.6
2010	116	4.9	0.6–8.0

The last round of MDA was performed in 2007. Since only selective treatment of MF carriers was performed in 2001, no Brugia Rapid tests were performed on the samples collected in 2002

DISCUSSION

The newly developed *Brugia RAPID* test for the detection of *B. malayi* infection was evaluated using 753 sera from 6 institutions. Encouraging results of this first evaluation study showed: 99% specificity, 97% sensitivity, 99% negative predictive value and 97% positive predictive value.

Our study demonstrated the high specificity & sensitivity of the *Brugia RAPID* test. Therefore, this is a promising tool to assist in the brugian filariasis elimination programme.



Mass Drug Administration (MDA)

Countries that undertook mass drug administration using Brugia RAPID test:

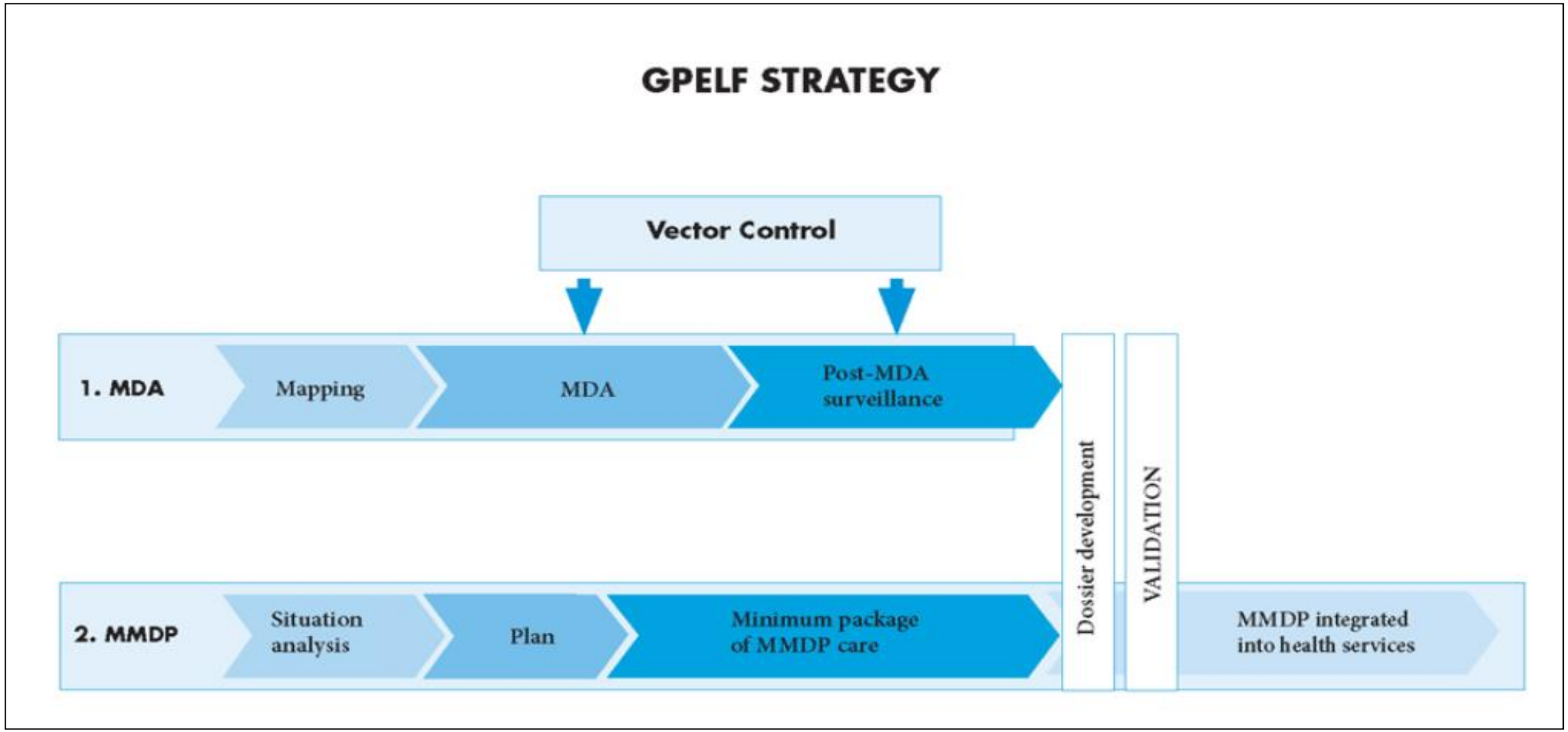
1. Malaysia
2. Indonesia
3. Philippines
4. Vietnam
5. Cambodia
6. Laos
7. Myanmar
8. Polynesia
9. China
10. India
11. Egypt
12. Sub-Saharan African countries (e.g. Tanzania)

Mass Drug Administration (MDA)

Annual single doses of diethylcarbamazine either alone or combined with albendazole are effective in lowering microfilaria (mf) load in the infected communities. Such annual single dose therapy when repeated is capable of keeping mf levels very low.

The Global Programme for Elimination of Lymphatic Filariasis (GPELF) recommends mass treatment of endemic populations using repeated annual single doses for 5-6 years to interrupt transmission and institution of morbidity management for affected individuals. This programme is currently in place in many countries including those endemic for *Brugia malayi* LF.

Global Programme to Eliminate Lymphatic Filariasis (GPELF)



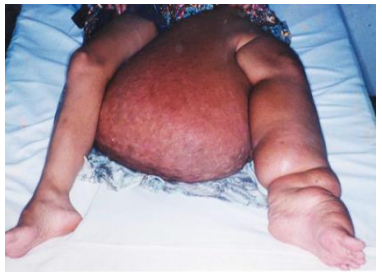
DISCUSSION

The *Brugia RAPID* dipstick test which uses filaria-specific IgG4 antibodies detection based on BmR1 recombinant antigen has been widely used to monitor the mass drug administration (MDA) program by the World Health Organization (WHO).

CONCLUSION

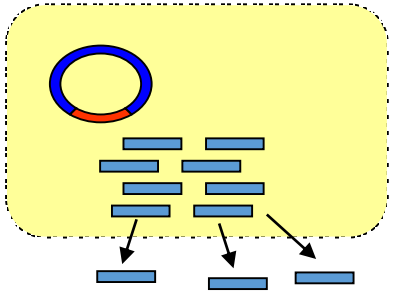
The use of *Brugia RAPID* test has significantly contributed in the translational efforts of mass drug administration that aimed to eliminate LF and thus improving human health worldwide.

TRANSLATIONAL MEDICINE

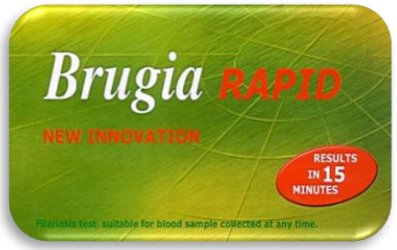


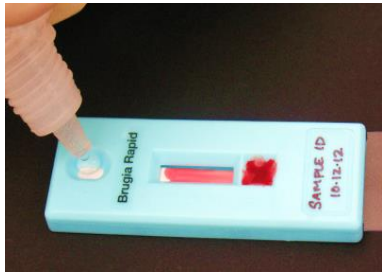
The Disease

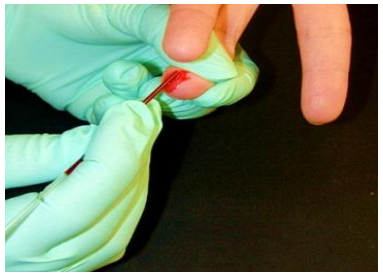
Fundamental Research



Applied Research







Use of Brugia RAPID in WHO's Mass Drug Administration

Community

World Health Organization

MMDP

Thank You

RESOLUTIONS

1. Basic science research is important in providing reliable results that can be used in clinical application as well as the advancement of scientific community and the public.

2. Findings of the basic science research need to be translated to help the community.

3. The outcomes of interest for translational medicine need to be carefully defined and a balance must be created between the needs of healthcare consumers and health outcomes.

4. Development of simple, practical and safe intervention, such as ***Brugia RAPID*** **test**, is a target for translational research.