A miniPCR-duplex Lateral Flow Dipstick Platform in Combination with the Microfluidic Device for Rapid and Visual Diagnosis of Lymphatic Filariae Infection

Achinya Phuakrod
Mahidol University, Faculty of Medicine Siriraj HospitalLibrary and Knowledge Center: Mahidol University

Witsaroot Sripumkhai
National Electronics and Computer Technology Center

Wutthinan Jeamsaksiri
National Electronics and Computer Technology Center

Pattaraluck Pattamang
National Electronics and Computer Technology Center

Sumart Loymek
Department of Disease Control,

Paul J. Brindley
George Washington, School of Medicine and Health Science

Patsharaporn T. Sarasombath
Mahidol University Faculty of Medicine Siriraj Hospital

Sirichit Wongkamchai (sirichit.won@mahidol.ac.th)
Mahidol University Faculty of Medicine Siriraj Hospital

Research Article

Keywords: Brugia malayi, DLFD, duplex lateral flow dipstick, lymphatic filariasis, miniPCR, microfluidic, Wuchereria bancrofti

Posted Date: April 30th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-446171/v1

Abstract

Background: Lymphatic filariasis is a major neglected tropical disease that is a leading cause of permanent and long-term disability worldwide. Significant progress made by the Global Programme to Eliminate Lymphatic Filariasis (GPELF) has led to a substantial decrease in the levels of infection. Accordingly, access is now needed to a cost-efficient, non-laborious diagnostic assay(s) capable of detecting low levels of microfilariae and without the need for infrastructure and expensive equipment.

Methods: We developed a diagnostic assay that combines a miniPCR with a duplex lateral flow dipstick (DLFD) assay. The design of the PCR primers was guided by sequence alignments of the *Hha*I and *Ssp*I genes of *Brugia malayi* and *Wuchereria bancrofti*, respectively. The limits of detection, accuracy, and cross-reactivity of the assay were evaluated, after which the assay was deployed to assess infection using blood samples obtained from Myanmar study participants living along the Thailand-Myanmar border, a region in Tak province endemic *W. bancrofti*. In addition, blood samples were provided by from Thais in Narathiwat province residing in an area endemic for brugian filariasis. We also combined a previously reported semi-automated microfluidic device with the miniPCR-DLFD to facilitate rapid detection and species identification of microfilariae in human blood.

Results: The miniPCR-DLFD assay exhibited a detection limit of two microfilariae per mililiter blood sample, and cross-amplification was not observed with from other parasites. For field validation, microfilariae and DNA of *W. bancrofti* were detected from two (0.6%) and five (1.5%) out of 328 (100%) blood samples, respectively. For the Narathiwat samples, microfilariae and DNA of *B. malayi* were detected from one (0.46%) and two (0.9%) out of 216 (100%) blood samples, respectively. A rapid and visual of species identification was accurately obtained in all microfilariae entrapped by our previously developed microfluidic device.

Conclusion: A miniPCR-DLFD platform alone or coupled with the microfluidic device provided rapid and visual diagnosis of lymphatic filariasis. The microfluidic device, the miniPCR, and the DLFD are all portable. Coupled with a portable microcentrifuge for DNA extraction, this is a promising platform for the diagnosis of lymphatic filariasis in point-of-collection settings with a modest cost (~USD $ 5) per sample.

Introduction

Lymphatic filariasis (LF), caused by lymphatic parasites, *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*, is one of the most neglected tropical diseases. LF is a mosquito-borne disease and remains a major public health concern, especially in Asia, Africa, the Western Pacific, and parts of the Caribbean and South America [1–3].

In addition to Giemsa staining for microfilaria, a variety of detection methods such as antigen detection, antibody detection and molecular diagnosis have been developed to improve diagnostic performance and utility [4, 5, 6]. An immunochromatographic test (ICT) for the detection of *W. bancrofti* circulating antigen is available commercially [6]. ICTs are easy to perform and their results are easily interpreted.

Nevertheless, filarial antigenemia that persists for years, even after treatment, limits the value of ICTs, as evidenced by follow-up studies post-treatment [7–9]. A few articles have reported new tests that can detect circulating antigens from *Brugia malayi* in human blood. However, those tests have not yet been independently verified, and none are commercially available [10–14]. Antifilarial IgG4 antibody detection has also been developed [5, 6, 15], and it is a useful addition to the limited array of brugian filariasis diagnostic tools available. However, this antibody test does not discriminate between filarial species, and antibody titres fall only slowly following anthelmintic treatment [16].

As the Global Programme to Eliminate Lymphatic Filariasis moves towards the achievement of its goal, the prevalence of LF and the rates of disease transmission have decreased substantially [17, 18]. The success of the programme has led to dramatic reductions in both microfilaremia and antigenemia levels in those countries that have completed multiple rounds of mass drug administration [19]. Paradoxically, with the steady decline in the disease levels, there is an increasing need for more sensitive and specific diagnostic assays capable of detecting low levels of parasites [20–22]. In addition, proactive monitoring for LF in historically endemic areas is necessary in order to eliminate the disease [1, 17, 23]. Thus, there is a need to develop a reliable, rapid, user-friendly, less labour-intensive detection method of LF diagnosis that has the capability to screen for, and identify, causative species [4].

As to molecular diagnosis for LF, PCR assays have the advantage of detecting active infections. Moreover, PCR detects infections with nocturnally periodic strains of filariae in daytime blood collections [20, 22, 24], and PCR species identification is highly reliable [21, 24, 25]. The disadvantages of standard PCR are that it is time-consuming, requires dedicated laboratory instruments and reagents, and is available at only some facilities.

Several compact PCR-based methods and devices have been validated for use for point-of-collection detections [26, 27]. The miniPCR thermocycler represents an attractive and affordable device with the potential for use at collection sites in endemic-country settings. This user-friendly and portable instrument is commercially available at a modest price [26, 27]. However, the miniPCR still requires gel-analysis steps involving gel electrophoresis and imaging. In order to obviate these latter steps, several studies have proposed the use of a PCR-nucleic acid lateral flow immunochromatographic assay (PCR-NALFIA) using a lateral flow dipstick (LFD) [27, 28]. LFD-based assays can detect specific DNA products in as little as 10 minutes [27, 28].

The present study proposed for the first time to developed a miniPCR assay coupled with a duplex lateral flow dipstick (DLFD) to detect both *B. malayi* and *W. bancrofti* DNA in samples of human blood. We also applied this miniPCR-DLFD in combination with our recently developed semi-automated microfluidic device [29] to facilitate mass screening and rapid visual species identification of microfilariae of lymphatic filariae in human blood.

Materials And Methods

Ethics approval

Before commencement of the study, ethics approval was obtained from the Ethics Committee of the Faculty of Medicine Siriraj Hospital, Mahidol University (approval number, Si129/2016). The research also fully complied with the ethics principles and guidelines for human experimentation issued by the National Research Council of Thailand. The formal consent of the participants was obtained verbally.

Extraction of DNA from blood samples

DNA was extracted from 50 µL of ethylenediaminetetraacetic acid blood samples using the High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Penzberg, Germany) according to the manufacturer's protocol. Following extraction, the DNA was eluted in 100 µL of elution buffer and stored at -20º C until use. The DNA concentration was determined using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific Inc, Waltham, Mass., USA) before the DNA was subjected to PCR and miniPCR analysis.

Development of a miniPCR-DLFD

Primer design and optimization of standard PCR condition

The primer designs were based on alignments of the *Hha*I and *Ssp*I repetitive non-coding DNA sequences of *B. malayi* and *W. bancrofti*, respectively [30,31,32]. These regions contain species-specific sequences that are useful for identifying *B. malayi* and *W. bancrofti*. The *Hha*I and *Ssp*I sequences were obtained from the National Center for Biotechnology Information database (GenBank accession numbers M12691.1 for *B. malayi* and L20344.1 for *W. bancrofti*). Fig. 1 presents the sequences of the forward and reverse primers. A BLAST analysis (National Center for Biotechnology Information, Bethesda, Md., USA) was performed to check the specificity of the primers to the target sequences. The presence of the 145-bp and 182-bp amplification products of *Hha*I and *Ssp*I, respectively, was verified by 2% agarose gel electrophoresis.

The PCR amplifications were carried out in a volume of 20 µl, consisting of 10 µl of PCR master mix (Quantabio; Qiagen Beverly, LLC., Mass., USA); 0.2 µM of each forward and reverse primer; 7.2 µl of dH_2O; and 2 µl of DNA template. Nuclease-free water was used as a negative control. DNA from the blood samples containing mf of *B. malayi* and *W. bancrofti* were used as positive controls.

To optimize the PCR conditions, a gradient PCR was performed using the Veriti 96-Well Thermal Cycler (Applied Biosystems, Thermo Fisher Scientific Inc, Waltham, Mass., USA) with the annealing temperature ranging from 55–62º C for both sets of primers. At an annealing temperature of 56° C, both set of primers (*Hha*I and *Ssp*I) amplified clearly detectable products. The optimized amplification conditions for both

primer sets included an activation step at 95° C for 5 min, followed by a 30-step amplification of 30 s at 95° C, 30 s at 56° C, and 30 s at 70° C, with a last step at 70° C for 5 min.

Assay conditions to validate the miniPCR

The PCR assays for *B. malayi* and *W. bancrofti* were separately performed using a miniPCR instrument (DBA miniPCR bio; Amplyus LLC., Cambridge, Mass., USA), employing the same reagents and conditions used for the standard PCR, as noted above. For use in a DLFD, the 5’ ends of the designed forward primers of *Hha*I and *Ssp*I were labelled with fluorescein isothiocyanate (FITC). The 5’ ends of reverse primers of *Hha*I and *Ssp*I were labelled with digoxin (DIG) and biotin, respectively. The sequences of the primers and their corresponding amplicons are presented in Fig. 1.

Construction of DLFD for nucleic acid detection

A DLFD is composed of four parts: the sample pad, conjugate pad, nitrocellulose membrane and absorbent pad. The sample pad is pretreated with buffer, and it can offer suitable pH and ion strength for the detection. The conjugate pad is used for the storage of reporter molecules (colloidal gold conjugated mouse anti-FITC). For the development of our DLFD, anti-digoxin (anti-DIG; Test line 1) and streptavidin (Test line 2) were sprayed on the nitrocellulose membrane to create test zones using the IsoFlow Reagent Dispenser (Imagene Technology, Inc., Hanover, N.H., USA), whereas anti-mouse antibody was sprayed on the nitrocellulose membrane to form a control zone (control line) by the AirJet Quanti 3000 Nanoliter aerosol dispenser (BioDot, Inc., Irvine, Calif., USA). The membrane was then dried at 37° C for 12 hours. The nitrocellulose membrane was attached to the central part of an adhesive plate. The lateral flow dipstick was then assembled, as shown in Fig 2; a schematic illustration of the DLFD is also presented in that figure.

Optimization of anti-DIG and streptavidin concentration

To optimize the concentration of anti-DIG, various concentrations of anti-DIG (0.5 µg/strip, 0.75 µg/strip or 1 µg/strip; Test line 1) were sprayed on the nitrocellulose membrane to optimize the concentration of anti-DIG, using the IsoFlow Reagent Dispenser (Imagene Technology, Inc.). The concentrations were at 1 µl per mm. The lateral flow dipstick was then assembled. Various concentrations of streptavidin (i.e., 0.5 µg/strip, 0.75 µg/strip, or 1 µg/strip) (Test line 2) were sprayed on the nitrocellulose membrane to optimize concentration of streptavidin using the AirJet Nanoliter aerosol dispenser (BioDot, Inc.). The assay was performed using positive (DNA of *B. malayi*) and negative (dH_2O) controls.

DLFD for detection of amplification products

To detect the amplification products of each sample, 1 µl of amplification product from each set of primers (specific to *Hha*I and *Ssp*I) was added into a well of the 96-well plate containing 100 µl of sample buffer. The dipstick was placed into the well vertically, and the reaction was read within 5–10 min. The appearance of positive, pink-coloured lines was observed using the naked eye on both the test and control lines. With respect to negative results, the pink-coloured line was apparent solely on the control line.

The miniPCR-DLFD specificity

To verify the specificity of the miniPCR-DLFD-based detection platform, genomic DNA samples isolated from 11 other parasites—*Trichinella spiralis*, *Angiostrongylus cantonensis*, *Gnathostoma spinigerum*, *Enterobius vermicularis*, *Necator americanus*, *Taenia solium*, *Plasmodium falciparum*, *Litomosoides sigmodontis*, *Brugia pahangi*, *Dirofilaria immitis* and *D. repens*—were used to measure off-target PCR amplification by the miniPCR-DLFD. The amount of DNA used for the miniPCR of each parasite was 20 ng/reaction.

Detection limit of miniPCR-DLFD

To study the detection limit of the miniPCR-DLFD, mf of *B. malayi* was spiked into ethylenediaminetetraacetic acid blood specimens obtained from healthy subject, as listed in Table 1. Four sets of the samples were prepared (sample IDs 1–24). The DNA of *B. malayi* as well as negative blood samples were used as positive and negative controls. DNA that had been extracted from the blood samples underwent amplification using the standard PCR as well as the miniPCR, followed by DLFD assay.

Comparison of the Giemsa-stained thick blood smears, standard PCR based amplification and the miniPCR-DLFD assay

The study population consisted of 10 subjects positive for *B. malayi* mf, 14 subjects positive for *W. bancrofti* mf, and 50 healthy controls. Blood was also collected from the 14 *W. bancrofti* mf positive subjects on day 7 following treatment with diethylcarbamazine and albendazole treatment (Table 2). Giemsa staining of thick blood smears, standard PCR amplification, and miniPCR-DLFD assays were performed.

Field validation

For field validation of the miniPCR-DLFD, a total of 216 blood samples were collected from Thai residents in Narathiwat Province, an area endemic for brugian filariasis. A further 328 blood samples were obtained from Burmese immigrants who resided along the Thailand–Myanmar border in Tak Province, which is a region endemic for *W. bancrofti* (Table 2). All of the blood samples from these study participants were collected during the daytime.

Microfluidic device coupled with miniPCR-DLFD

Detection of microfilariae was undertaken in all study samples using the microfluidic device, as described [29]. Briefly, 50 µl of blood was suspended in 150 µl of lysis buffer A, mixed, and then incubated at room temperature for 10 min. The sample was uploaded for analysis by drawing buffer B, 15 µl, into a one ml syringe that connects to the adapter and to the inlet port. The pump was started and the sample solution introduced into the microfluidic device via the inlet port. The microfilariae were trapped in the microfluidic chip while the remaining solution exited the microfluidic chip via the outlet port into the waste tube. The trapped microfilariae were inspected by light microscopy at 10x magnification. To identify species of the trapped mf in the microfluidic chips, 100 µl Tris-EDTA buffer was dispensed into the chip via the inlet port. The chip was placed on a hot plate at 56°C for 15 min, after which the solution containing the trapped mf was withdrawn through the outlet port and transferred to a 1.5 ml Eppendorf tube. The tube was subjected to centrifugation at 15,520× g for 10 min. The supernatant was discarded, and DNA was extracted from the pelleted material using the Roche high pure PCR template preparation kit, according to the manufacturer's instructions (Roche Diagnostics GmbH, Penzberg, Germany). Species identification was performed by *Hha*I/*Ssp*I gene amplification by miniPCR (Amplyus) followed by use of the DLFD testing using the above-mentioned miniPCR assay primers and employing the same reaction recipes and conditions as described above.

Results

Optimization of anti-DIG and streptavidin concentration

The optimal concentrations of anti-DIG and streptavidin which showed clearly visible results for test line 1 (*B. malayi*) and test line 2 (*W. bancrofti*) of the DLFD were 0.75 µg anti-DIG/strip and 1 µg streptavidin/strip, respectively. The optimal amount of PCR products from the miniPCR that yielded a clear band on the DLFD strip was 1 µl of amplification product from each set of primers (*HhaI* and *SspI*) in 100 µl of sample buffer. Clearly detectable, positive, pink-coloured lines were observed within 10 minutes.

The miniPCR-DLFD specificity

As illustrated in Fig. 3A, the DNA from 11 other parasites were all negative by the miniPCR-DLFD assay.

The detection limit of the miniPCR-DLFD

The miniPCR-DLFD still detected a positive band in the samples numbered 17–20, each of which contained 2 mf per millilitre of blood (Fig. 3A and Table 1).

Table 1

The sample ID, number of spiked microfilaria (mf) mf of *Brugia malayi* in a one millilitre blood sample, concentration of extracted DNA (ng/µL), and outcome of the miniPCR-DLFD assay and standard PCR with gel electrophoresis as positive (+) or negative (-).

Sample ID	Mf of *B. malayi* / mL blood	Extracted DNA (ng/µL)	miniPCR-DLFD	Standard PCR with gel electrophoresis
1	20	23	+	+
2	20	22.5	+	+
3	20	17.3	+	+
4	20	21	+	+
5	10	22.5	+	+
6	10	22.1	+	+
7	10	23	+	+
8	10	21.5	+	+
9	5	22.6	+	+
10	5	18	+	+
11	5	17.7	+	+
12	5	23.3	+	+
13	4	21.4	+	+
14	4	25.7	+	+
15	4	21.9	+	+
16	4	22.3	+	+
17	2	19.2	+	+
18	2	21.4	+	+
19	2	18.5	+	+
20	2	20.4	+	+
21	1	27.2	-	-
22	1	20.3	-	-
23	1	20.8	-	-
24	1	21.0	-	-

Comparison of the Giemsa-stained thick blood smears, standard PCR based amplification and the miniPCR-DLFD assay

For all 24 mf positive blood samples as well as the 50 controls tested, the standard PCR results agreed with the miniPCR results using both test strip-based detection and electrophoresis-based detection (Table 2). As to the 14 *W. bancrofti* mf positive subjects who received diethylcarbamazine and albendazole treatment, mf and DNA of *W. bancrofti* were detected in four of the 14 blood samples. Only DNA was detected in two of the post-anthelmintic treatment blood samples. No mf nor DNA of *W. bancrofti* were detected from the remainder of the samples (Table 2). Figure 4 presents the findings obtained with the tested samples.

Field validation

Regarding the field validation in Narathiwat Province, mf and DNA of *B. malayi* were detected in one of the 216 blood samples, whereas only DNA of *B. malayi* was detected in one of those samples. As to the 328 blood samples collected from Tak Province, mf and DNA of *W. bancrofti* were detected in two of the samples, while only DNA of *W. bancrofti* was detected in three of the samples (Table 2).

Table 2

Comparison of the standard PCR-based amplification, miniPCR-DLFD based amplification and detection of microfilariae (mf) detection by stained thick blood smear for diagnosis of infection with *Wuchereria bancrofti* and *Brugia malayi* in study blood samples

Study sample group	Number of samples	Thick blood smear staining for mf detection		Standard PCR with gel electrophoresis		miniPCR-DLFD	
		+	-	+	-	+	-
Samples from subject positive for mf of *B. malayi*	10	10	0	10	0	10	0
Samples from subject positive for mf of *W. bancrofti*	14	14	0	14	0	14	0
Samples from healthy controls	50	0	50	0	50	0	50
Post-treatment sample from subjects positive for *W. bancrofti* mf collected on day 7 after treatment with diethylcarbamazine and albendazole	14	4	10	6	8	6	8
Samples from field study in Narathiwat Province	216	1	215	2	214	2	214
Samples from field study in Tak Province	328	2	326	5	323	5	323

Microfluidic device coupled with the miniPCR-DLFD

After processing of the microfilaria detection using the microfluidic device, mf of the 24 mf-positive human blood samples were trapped in the detection zone within the microfluidic chips. Species of the entrapped mf were further identified by the miniPCR-DLFD. The 10 samples were identified as *B. malayi* mf, and 14 were confirmed to be *W. bancrofti* (Fig 5).

Discussion

In the present study, we developed a miniPCR-DLFD for rapid and visual diagnosis of lymphatic filariasis. The duplex LF detection is another format of lateral flow dipstick that can be used for detection of more than one target filarial species, and the assay is performed on a strip containing test lines equal to the number of target species to be analysed. In addition, DLFD exhibits increased sensitivity for PCR product detection compared with gel electrophoresis, and it takes less time [28, 33, 34].

The detection limit of the thick blood smear staining technique for mf detection is at least 16–20 mf in one ml of blood [24, 25]. The result obtained from the present study, as the detection limit of the miniPCR-DLFD was two mf of *B. malayi* per ml of blood sample. This is 10-fold more sensitive than thick blood smear staining. Moreover, almost all species and strains of lymphatic filariae worldwide display nocturnal periodicity/sub-periodicity, given that samples collected during the daytime show a reduced sensitivity for the detection of mf [8]. Notably, of all 14 cases from whom blood samples were collected post-treatment, *W. bancrofti* DNA was still detected in six infected study participants whereas mf was detected in only four cases. These data encourage the usage of the miniPCR-DLFD as a sensitive tool for follow-up after treatment.

A small, portable, miniPCR instrument was used in this study to replace the need for a bulkier, standard-sized thermocycler. The portable miniPCR from Amplyus used by the current investigation is diminutive in size (5.1 x 12.7 x 10.2 cm) and not heavy (450 g), and it only requires 100–240 V (AC) and 50–60 Hz, 90 W to perform a PCR run of 16 amplifications/round. Furthermore, its cost (~ US $800) is not prohibitive. Taken together, these features make it an attractive and reliable alternative tool for point-of-care. With respect to cost, the reagents for the DNA extraction and miniPCR steps cost about $3/sample, and one DLFD strip costs $2. Thus, the total cost of the miniPCR-DLFD assay is of the order of $5 per sample. One limitation of using the miniPCR-DLFD for point-of-collection settings, however, is that the DNA extraction steps require somewhat cumbersome instruments including a high-speed microcentrifuge. We suggest that use of a portable microcentrifuge, e.g., WiseSpin CF-10 Diahan-Scientific, which weights ~ 315 5 kg, and operates up to 13,500 rpm, can address this issue.

A miniPCR thermocycler has been used for multiple diagnostic approaches [26, 27, 35, 36]. In the arena of studies on lymphatic filariasis, Zaky et al. used the miniPCR (Amplyus LLC) combined with a lateral flow strip for the rapid detection of amplification products of *Brugia* larvae origin in mosquitoes and they highlighted its utility as a backpack-portable, point-of-collection diagnostic platform [26]. By comparison,

our developed duplex lateral flow dipstick (DLFD) can detect amplification product of both *B. malayi* and *W. bancrofti* microfilariae from human blood sample, in a single strip.

We have previously developed a semi-automated microfluidic device for rapid, higher-throughput detection of microfilariae in animal blood. We next]performed a real time PCR with high resolution melting analysis (HRM-real time PCR) for species identification of the trapped mf [29]. The efficiency of the device was evaluated in laboratory and it was applied to study the prevalence of canine and feline microfilaremia [29, 37]. In this study, we applied the microfluidic device to detect microfilariae of *B. malayi* and *W. bancrofti* in human blood. We also replaced the HRM-real time PCR with the miniPCR-DLFD for the species identification step in order to reduce the need for infrastructure and expensive equipment. We posit that use of the microfluidic device, which is portable and weights only 3 kg, coupled with the miniPCR-DLFD can enable population-scale, mass screening for LF in endemic areas.

Conclusions

To overcome the impediments to facilitate rapid diagnosis for LF at the collection site, associated with infrastructure and expensive equipment, we now propose new diagnostic methods: i) a miniPCR-DLFD platform, and ii) a combination of microfluidic device with the miniPCR-DLFD for point-of-collection detection of *B. malayi* and *W. bancrofti* DNA that costs only USD $5 per sample. The miniPCR-DLFD alone or the combination of the microfluidic device with the miniPCR-DLFD represents a promising platform for the diagnosis of lymphatic filariae in point of collection settings.

Declarations

Acknowledgments

We thank the staff of the Phikhunthong Royal Project, Narathiwat Province, Thailand. We also thank Miss Bungon Sermsart and Dr. Anchalee Tungtrongchitr, Department of Parasitology, Faculty of Medicine, Siriraj Hospital, Mahidol University, for research assistance and support, and for a visiting scholar award to PJB.

Funding

This work was supported by the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (grant no. RO15936006; SW) and by the National Science and Technology Development Agency, The Ministry of Science and Technology, Bangkok, Thailand (grant no. P1551260; AP and WJ). Additional support was provided by the Thailand Graduate Institute of Science and Technology, National Science and Technology Development Agency, Thailand (grant no. SCA-CO-2561-7118TH).

Availability of data and materials

Supplement Fig. 1

Authors' contributions

SW participated in conceptualization, methodology, writing (review and editing); PTS participated in conceptualization, methodology, writing (review and editing); AP participated in conceptualization, methodology, blood collection, writing (first draft); WS participated in the design and production of the microfluidic chip; WJ participated in in the design and production of the microfluidic chip; PP participated in production of the microfluidic chip; SL participated in Blood collection and fieldwork; PJB participated in editing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Before commencement of the study, ethics approval was obtained from the Ethics Committee of the Faculty of Medicine Siriraj Hospital, Mahidol University (approval number, Si129/2016). The research also fully complied with the ethics principles and guidelines for human experimentation issued by the National Research Council of Thailand. The formal consent of the participants was obtained verbally.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. [2]Thai Microelectronic Center, National Electronics and Computer Technology Center, Thailand Science Park, Pathumthani, Thailand. [3]Office of Disease Prevention and Control, Region 12, Department of Disease Control, The Ministry of Public Health, Songkhla, Thailand. [4]Department of Microbiology, Immunology & Tropical Medicine & Research Center for Neglected Diseases of Poverty, School of Medicine & Health Sciences, George Washington University, Washington, DC, USA. [5]Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.

References

1. Lymphatic filariasis. [cited 18 Nov 2020]. Available: https://www.who.int/news-room/fact-sheets/detail/lymphatic-filariasis.
2. CDC-Centers for Disease Control and P. CDC—Lymphatic Filariasis—Resources for Health Professionals—Guidance for Evaluation and Treatment. 26 Oct 2020 [cited 17 Nov 2020]. Available: https://www.cdc.gov/parasites /lymphaticfilariasis/health_professionals/dxtx.html
3. Whitworth J, Hewitt K. Filariasis. Medicine. 2005; 1;33(8):61–4.
4. Riches N, Badia-Rius X, Mzilahowa T, Kelly-Hope LA. A systematic review of alternative surveillance approaches for lymphatic filariasis in low prevalence settings: Implications for post-validation settings. PLoS Neglected Tropical Diseases. 2020;14. doi:10.1371/journal.pntd.0008289.
5. Chanteau S, Moulia-Pelat J, Glaziou P, Nguyen N, Luquiaud P, Plichart C, et al. Og4C3 circulating antigen: a marker of infection and adult worm burden in Wuchereria bancrofti filariasis. J Infect Dis 1994;170 (1):247-250. doi:10.1093/infdis/170.1.247.
6. Melrose W, Durrheim D, Burgess G. Update on immunological tests for lymphatic filariasis. Trends in parasitology. Trends Parasitol;2004:20 (20):255-7. doi:10.1016/j. pt.2004.04.002.
7. Weil GJ, Sethumadhavan KV, Santhanam S, Jain DC, Ghosh TK. Persistence of parasite antigenemia following diethylcarbamazine therapy of bancroftian filariasis. Am J Trop Med Hyg. 1988;38:589–95. doi: 10.4269/ajtmh.1988.38 (3).589.
8. McCarthy JS, Zhong M, Gopinath R, Ottesen EA, Williams SA, Nutman TB. Evaluation of a polymerase chain reaction-based assay for diagnosis of *Wuchereria bancrofti* J Infect Dis. 1996;173 (6):1510–4. doi:10.1093/infdis/
9. Nicolas L, Plichart C, Nguyen LN, Moulia-Pelat JP. Reduction of Wuchereria bancrofti adult worm circulating antigen after annual treatments of diethylcarbamazine combined with ivermectin in French Polynesia. J Infect Dis. 1997;175 (2) 9:489–92.
10. Abdullah et al., 1993; Abdullah WO, et al. Detection of circulating antigens and parasite specific antibodies in filariasis. The Southeast Asian journal of tropical medicine and public health. 1993;24 (Suppl 2):31–6.
11. Lalitha P, et al. Development of antigen detection ELISA for the diagnosis of brugian and bancroftian filariasis using antibodies to recombinant filarial antigens Bm-SXP-1 and Wb-SXP-1. Microbiology and immunology 2002;46 (5):327–32.
12. Pandey V, et al. Antigen detection assay with parasite specific monoclonal antibodies for diagnosis of lymphatic filariasis. Clinica chimica acta; international journal of clinical chemistry 2011;412 (19-20):1867–73.
13. Wongkamchai S, et al. An antigen detection assay for diagnosing filariasis. Asian Pacific journal of allergy and immunology 2003;21 (4):241–51.
14. McNulty N, Mitreva M, Gary J Weil, Fischer Inter and intra-specific diversity of parasites that cause lymphatic filariasis. Infect Genet Evol Actions 2013;14:137–46. doi: 10.1016/j.meegid.2012.11.002.

15. Reddy M, Malhotra A, Harinath B. Detection of circulating antigen in bancroftian filariasis by sandwich ELISA using filarial serum IgG. J Helminthol 1984;58 (3):259-62. doi:10.1017/s0022149x00027103.
16. Helmy H, Weil GJ, Ellethy AS, Ahmed ES, Setouhy ME, Ramzy RM. Bancroftian filariasis: effect of repeated treatment with diethylcarbamazine and albendazole on microfilaraemia, antigenaemia and antifilarial antibodies. Trans R Soc Trop Med Hyg. 2006;100 (7):656–62.
17. Ichimori K, King JD, Engels D, Yajima A, Mikhailov A, Lammie P, et al. Global Programme to Eliminate Lymphatic Filariasis: The Processes Underlying Programme Success. PLOS Neglected Tropical Diseases. 2014;8 (12):e3328. doi:10.1371/ journal.pntd.0003328
18. Ramaiah KD, Ottesen EA. Progress and Impact of 13 Years of the Global Programme to Eliminate Lymphatic Filariasis on Reducing the Burden of Filarial Disease. PLOS Neglected Tropical Diseases. 2014;8 (11):e3319. doi:10.1371/journal.pntd.0003319
19. Gass K, Beau de Rochars MV, Boakye D, et al. A multicenter evaluation of diagnostic tools to define endpoints for programs to eliminate bancroftian filariasis. PLoS Negl Trop Dis. 2012;6(1):e1479. doi:10.1371/journal.pntd.0001479
20. Pilotte N, Torres M, Tomaino F, Laney S, Williams S. A TaqMan-based multiplex real-time PCR assay for the simultaneous detection of Wuchereria bancrofti and Brugia malayi. Molecular and biochemical parasitology. 2013;189 (1-2). doi:10.1016/ j.molbiopara.2013.05.001
21. Fischer P, Supali T, Wibowo H, Bonow I, Williams S. Detection of DNA of nocturnally periodic Brugia malayi in night and day blood samples by a polymerase chain reaction-ELISA-based method using an internal control DNA. The American journal of tropical medicine and hygiene. 2000;62 (2). 291-6. doi:10.4269/ajtmh.2000.62.291
22. Plichart C, Lemoine A. Monitoring and evaluation of lymphatic filariasis interventions: an improved PCR-based pool screening method for high throughput Wuchereria bancrofti detection using dried blood spots. BioMed Central 2013;6 (1):1–5. Report No.: 1. Available: https://parasitesandvectors.biomedcentral.com/articles/ 10.1186/1756–3305–6–110
23. Srividya A, Subramanian S, Jambulingam P, Vijayakumar B, Raja JD. Mapping and monitoring for a lymphatic filariasis elimination program: a systematic review. Research and Reports in Tropical Medicine. 2019;10: 43. doi:10.2147/RRTM.S134186
24. Lizotte MR, Supali T, Partono F, Williams SA. A Polymerase Chain Reaction Assay for the Detection of *Brugia Malayi* in Blood. The American Journal of Tropical Medicine and Hygiene. 1994;51(3):314–21. doi:10.4269/ajtmh.1994.51.314
25. Ottesen E, Duke B, Karam M, Behbehani K. Strategies and tools for the control/elimination of lymphatic filariasis. Bulletin of the World Health Organization. 1997;75 (6):491. Available: https://pubmed.ncbi.nlm.nih.gov/9509621/
26. Zaky W, Tomaino F, Pilotte N, Laney S, Williams S. Backpack PCR: A point-of-collection diagnostic platform for the rapid detection of Brugia parasites in mosquitoes. PLoS neglected tropical diseases. 2018;12 (11). doi:10.1371/journal.pntd.0006962

27. González-González E, Jl M-R, Pedroza S, Cuellar-Monterrubio A, Márquez-Ipiña A, Lira-Serhan D, et al. Validation of use of the miniPCR thermocycler for Ebola and Zika virus detection. PLoS One; 2019;14 (5):e0215642. doi:10.1371/journal.pone.0215642
28. Pecchia S, Da Lio D. Development of a rapid PCR-Nucleic Acid Lateral Flow Immunoassay (PCR-NALFIA) based on rDNA IGS sequence analysis for the detection of Macrophomina phaseolina in soil. J Microbiol Methods 2018;151:118-28. doi:10.1016/j.mimet.2018.06.010.
29. Phuakrod A, Sripumkhai W, Jeamsaksiri W, Pattamang P, Juntasaro E, Thienthong T, et 478 al. Diagnosis of feline filariasis assisted by a novel semi-automated microfluidic device 479 in combination with high resolution melting real-time PCR. Parasites Vectors. 2019;12 (1):159. doi:10.1186/s13071-019-3421-z.
30. Chansiri K, Tejangkura T, Kwaosak P, Sarataphan N, Phantana S, Sukhumsirichart W. PCR based method for identification of zoonostic Brugia malayi microfilariae in domestic cats. Molecular and cellular probes. 2002;16. doi:10.1006/mcpr.2001.0402
31. Mishra K, Raj D, Hazra R, Dash A, Supakar P. The development and evaluation of a single step multiplex PCR method for simultaneous detection of Brugia malayi and Wuchereria bancrofti. Molecular and cellular probes. 2007;21 (5-6). doi:10.1016/j.mcp.2007.05.001
32. Triteeraprapab S, Kanjanopas K, Suwannadabba S, Sangprakarn S, Poovorawan Y, Scott AL. Transmission of the nocturnal periodic strain of Wuchereria bancrofti by Culex quinquefasciatus: establishing the potential for urban filariasis in Thailand. Epidemiology and Infection. 2000;125 (1):207. doi:10.1017/s0950268899004355
33. Sajid M, Kawde A-N, Daud M. Designs, formats and applications of lateral flow assay: A literature review. Journal of Saudi Chemical Society. 2015;19 (6):689–705. doi:10.1016/j.jscs.2014.09.001
34. Xu Y, Liu Y, Wu Y, Xia X, Liao Y, Li Q. Fluorescent probe-based lateral flow assay for multiplex nucleic acid detection. Anal Chem 2014;86 (12):5611-4. doi:10.1021/ac5010458
35. Boguraev A-S, Christensen HC, Bonneau AR, Pezza JA, Nichols NM, Giraldez AJ, et al. Successful amplification of DNA aboard the International Space Station. NPJ Microgravity. 2017;16:26. https://doi.org/10.1038/s41526–017–0033–9
36. González-González E, Trujillo-de Santiago G, Lara-Mayorga IM, Martínez-Chapa SO, Alvarez MM. Portable and accurate diagnostics for COVID-19: Combined use of the miniPCR thermocycler and a well-plate reader for SARS-CoV-2 virus detection. PLoS One. 2020;15(8):e0237418. doi: 10.1371/journal.pone.0237418.
37. Loymek S, Phuakrod A, Zaelai K, Sripumkhai W, Vongjaroensanti P, Wongkamchai S. Investigation on the Prevalence of Canine Microfilaremia in Thailand Using a Novel Microfluidic Device in Combination with Real-Time PCR. Vet Sci. 2021;8 (3):39. https://doi.org/10.3390/vetsci8030039

Figures

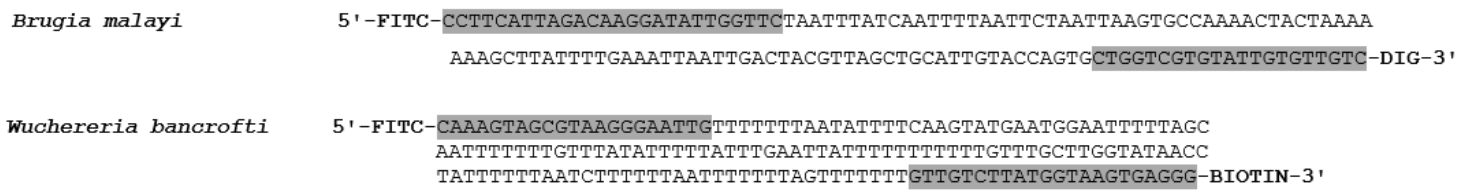

Figure 1

Alignment of HhaI repetitive non-coding DNA sequence of Brugia malayi (GenBank accession number M12691.1; position 132-277) and SspI repetitive non-coding DNA sequence of Wuchereria bancrofti (GenBank accession number L20344.1, positions 13- 195). Gray areas indicate primers. For the DLFD, the HhaI and SspI primers were labelled with fluorescein isothiocyanate (FITC) and digoxin (DIG), respectively.

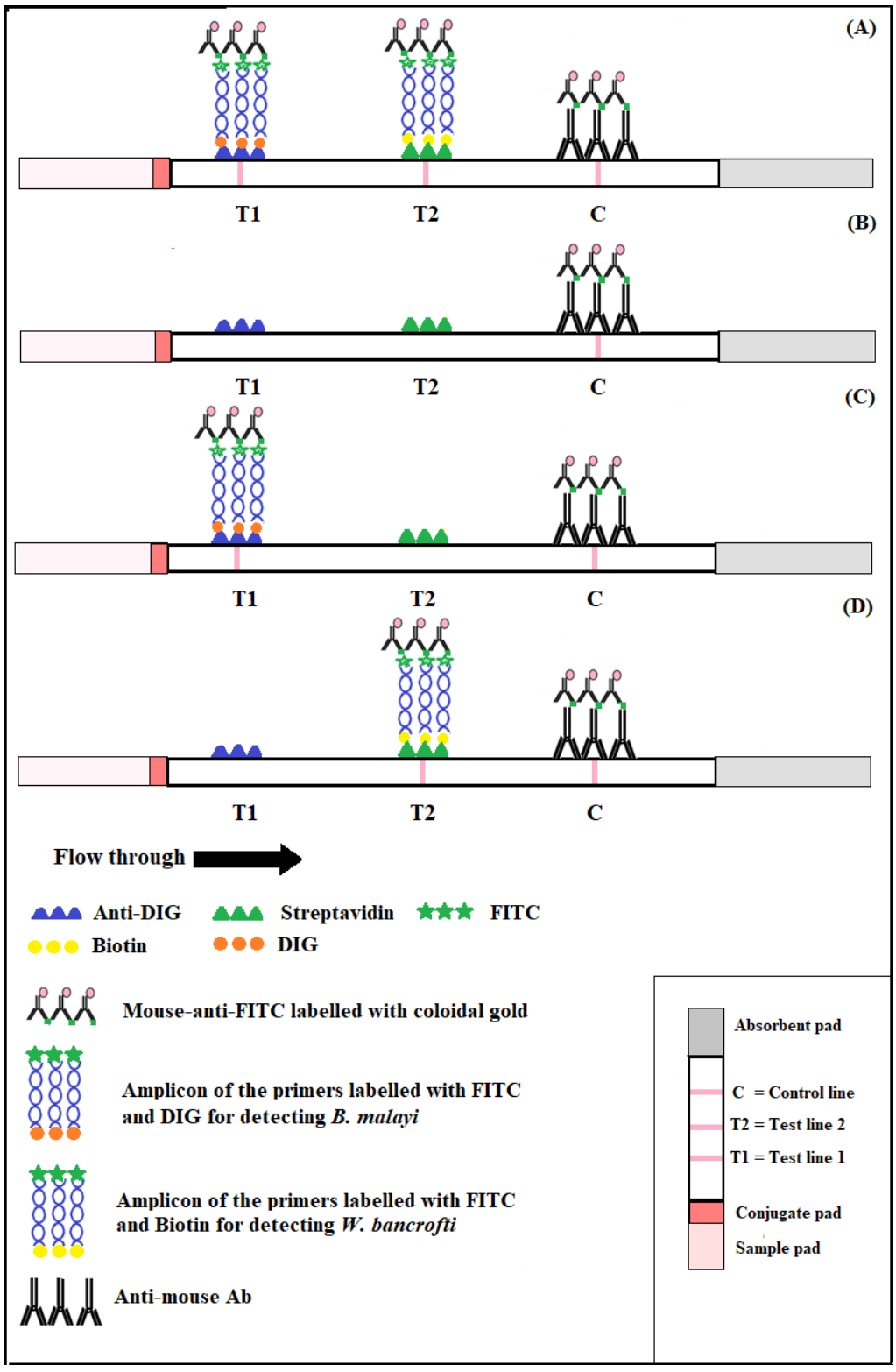

Figure 2

Schematic illustration of the duplex lateral flow dipstick (DLFD) for two target detections. T1 = test line 1 for detecting Brugia malayi; T2 = test line 2 for detecting Wuchereria bancrofti; and C = control line. (A) = positive control; (B) = negative control; (C) = positive for W. bancrofti; and (D) = positive for B. malayi.

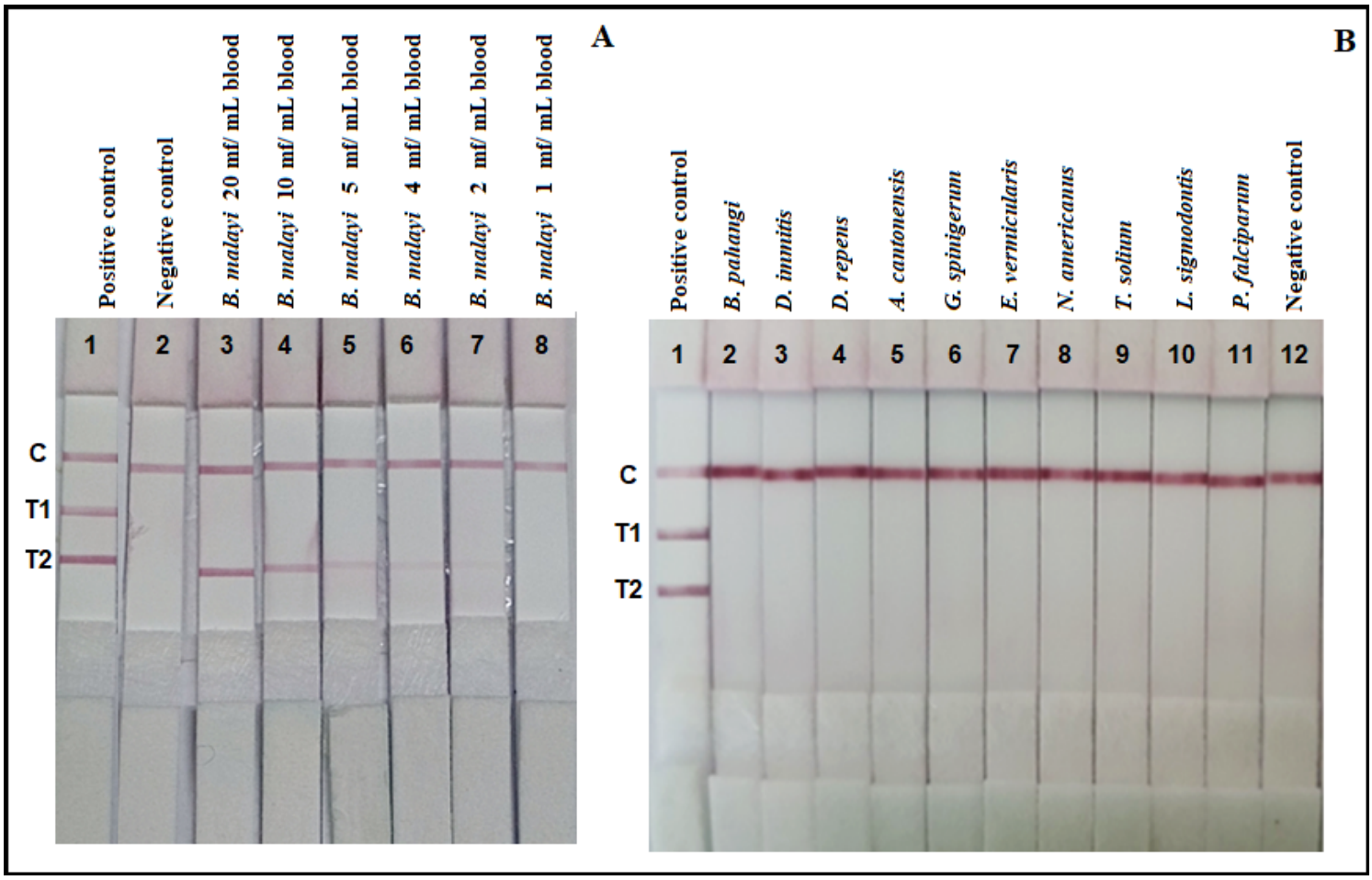

Figure 3

The detection limit of the miniPCR-DLFD using blood samples spiked with various numbers of B. malayi mf per mL blood (A), and the specificity of the miniPCR-DLFD verified with DNA templates of other parasites (B). T1 = detection zone for Brugia malayi DNA; T2 = detection zone for Wuchereria bancrofti DNA; and C = control line.

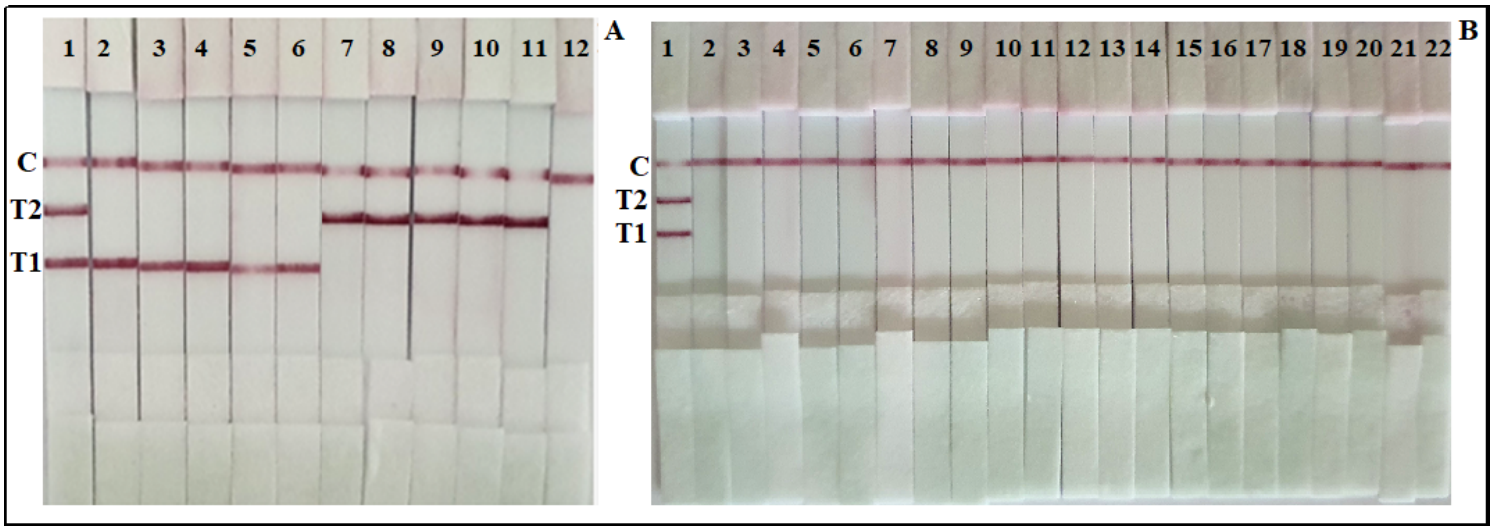

Figure 4

The miniPCR-DLFD assay validation. Reaction of amplicons in the DLFD assay. T1 = detection zone for Brugia malayi DNA; T2 = detection zone for Wuchereria bancrofti DNA; C = control line. (A): lane 1 = positive control; 2–6 = B. malayi mf-positive blood samples; 7–11 = W. bancrofti mf-positive blood samples; and 12 = negative control. (B): lane 1 = positive control; 2–21 = normal blood samples; and 12 = negative control.

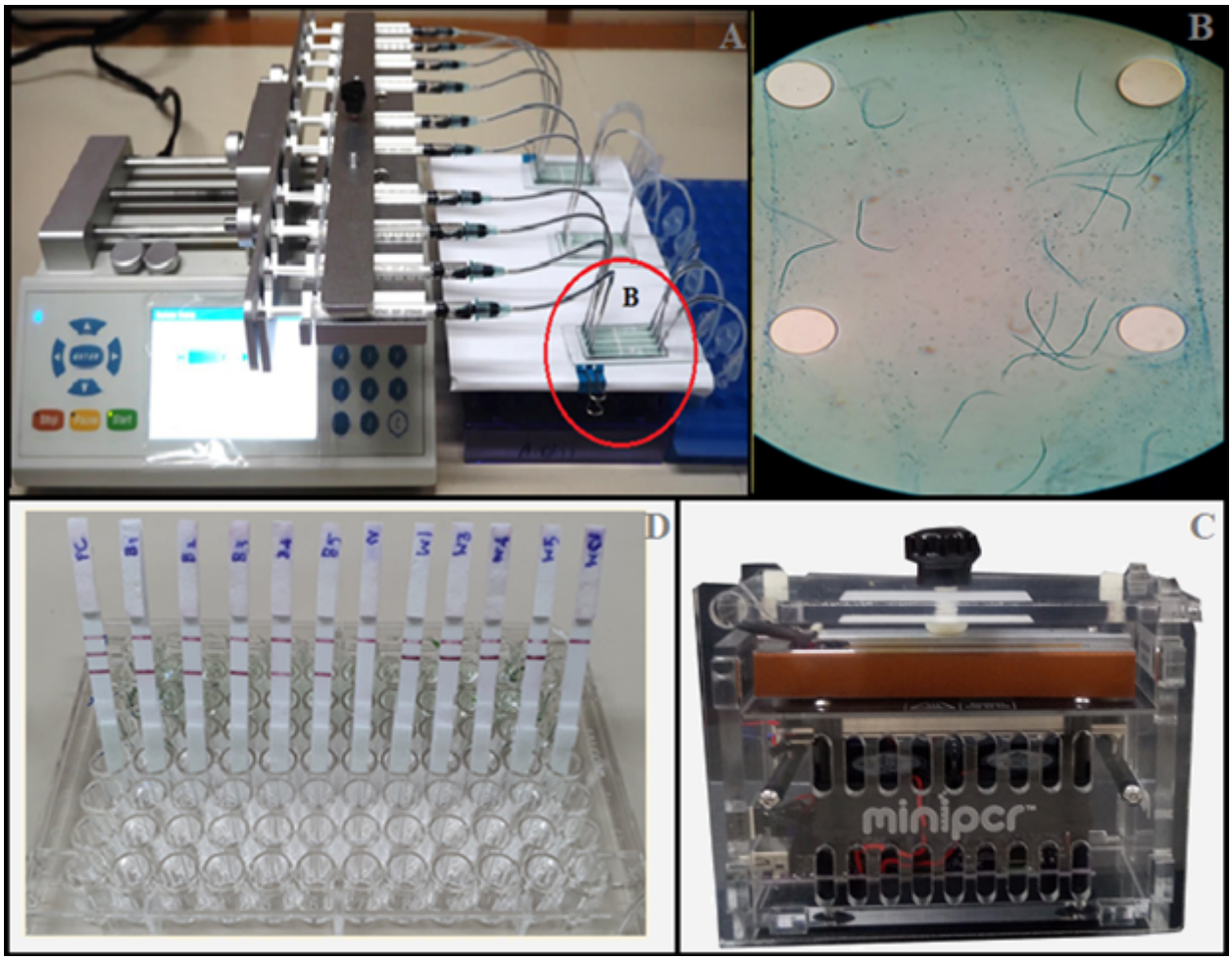

Figure 5

Microfluidic device coupled with the miniPCR-DLFD for rapid detection and identification of microfilariae of lymphatic filariae in human blood samples. (A) Microfluidic device. (B) Microfilariae trapped in the microfluidic chip (arrows). (C) A the miniPCR, and (D) Representative DLFD shows reaction of amplicon of the trapped microfilariae; strip 1: positive control containing W. bancrofti and B. malayi DNA, strips 2-11: representative of test samples, strip 12: negative control.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Figure1supplement.pdf
- Graphicalabstract.tif