



Immunochromatographic thread-based test platform for diagnosis of infectious diseases

Misago Seth^{1,3} · Daniel Mdetele² · Joram Buza¹

Received: 23 November 2017 / Accepted: 26 March 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

Patterning is an important step in fabrication of multiplexed microfluidic devices. Various approaches including cutting, photolithography, wax-printing, plotting and etching have been developed and tested. Recently, using threads has emerged as a convenient and low-cost approach for fabrication of microfluidic devices. We explored the application of threads in combination with nitrocellulose membrane to fabricate multi-channel immunochromatographic diagnostic devices. Microfluidic channels were made using hydrophilic threads and nitrocellulose membrane strips. Household sewing needle was used to weave hydrophilic thread into desired patterns through a double-sided mounting tape. Glass fibre discs were used as conjugate pads while nitrocellulose membrane was used for immobilisation of capture antibodies. Patterned threads were linked to nitrocellulose membrane strips by overlapping so that reagents flowing through threads were eventually transferred to the membrane. The design was tested using IgG, *H. pylori* and Hepatitis B surface antigen. Continuous flow was observed from hydrophilic threads to the nitrocellulose membrane, and a positive signal was visualised on the membrane within 5 min of sample application. The observed limit of detection ranged between 30 and 300 ng/ml for *H. pylori* and Hepatitis B, respectively. Using thread and tape offers a promising alternative for patterning of simple, low-cost multiplexed microfluidic diagnostic devices with potential point-of-care applications in resource-limited settings.

Keywords Immunochromatographic · Thread-based diagnostics · Infectious diseases · Multiplex microfluidic devices · Point-of-care · Low-cost diagnostics

1 Introduction

Membrane-based immunochromatographic lateral flow tests (ILFTs) are the first and most widely applied point-of-care tests currently in use (Boisen et al. 2015). These tests have wide applications ranging from the earliest pregnancy test strips (May 1991) to include sperm quality testing (Matsuura et al. 2014), infectious diseases diagnosis for medical and

veterinary fields (Nielsen et al. 2008; Rohrman et al. 2012; Kamphée et al. 2015), food safety, e.g. detection of mycotoxins, drug residues, food allergens, food-borne pathogens (Shankar et al. 2010; Toldra and Reig 2006; Law et al. 2014; Weng et al. 2016), urinalysis for non-infectious health biomarkers, e.g. detection of glucose, proteins, ketones, drugs of abuse, nitrite, bilirubin (Reches et al. 2010; Li et al. 2010; Martinez et al. 2007), bio-threats such as detection of anthrax, plague, tularemia, nerve agents, ricin and botulism (Zasada et al. 2015; Gessler et al. 2007; Shyu et al. 2002), environmental safety monitoring, e.g. monitoring chemical or microbial contaminations in soil, water and air (Hossain et al. 2012; Sicard et al. 2015).

Typically, a test strip consists of the following components: *Sample pad*; usually a filter paper or glass fibre pad onto which the test sample is applied, *Conjugate or reagent pad*; this contains antibodies specific to the target analyte conjugated to coloured particles (usually colloidal gold particles, or latex microspheres), *Reaction membrane*; typically a hydrophobic nitrocellulose or cellulose acetate membrane

✉ Misago Seth
sethm@nm-aist.ac.tz

¹ Global Health and Biomedical Sciences, School of Life Sciences and Bioengineering, Nelson Mandela African Institution of Science and Technology, P.O.Box 447, Arusha, Tanzania

² Zonal Veterinary Centre, Central Zone, P.O.Box 1752, Dodoma, Tanzania

³ Laboratory Sciences Department, National Institute for Medical Research, Tanga Centre, P.O.Box 5004, Tanga, Tanzania

onto which anti-target analyte and control antibodies are immobilised in a line that crosses the membrane to act as a capture zone or test line, and a *Wick or waste reservoir*; an absorbent pad designed to draw the sample across the reaction membrane by capillary action and collect excess reagents and buffer.

The sample pad acts as deposition site for the sample and may also help in filtration of particulate matters from the sample which passes through this pad towards distal sections of the strip. The conjugate pad holds dried detection antibodies conjugated to a reporter (like gold nanoparticle) until when re-dissolved by the sample and buffer. However, the reaction membrane binds protein more strongly so that even after the sample and buffer flows through the test and control zones, immobilised capture reagents (antibodies/antigens) are not dislodged, hence forming a focal point for signal development. This format of diagnostic meets the minimum criteria set by the world health organisation (WHO) for ideal diagnostics in developing nations which requires such diagnostics to be affordable, sensitive, specific, user-friendly, robust, equipment free, and deliverable to end users, abbreviated as ASSURED diagnostics (Urdea et al. 2006; Peeling et al. 2006).

Designing multiplexed membrane-based tests involves patterning of the sample and/or conjugate pads, and the membrane itself to create channels that direct reagents towards the multiple testing zones/points. The most common patterning methods are wax-printing for conjugate and sample pads (Carrilho et al. 2009; Lu et al. 2009) and laser cutting for conjugate pads and detection membrane (Fenton et al. 2009). Although these approaches are relatively simple to execute and are very promising in development of ASSURED diagnostics, it may still be difficult in developing countries to obtain the required equipment/machines such as laser cutter, solid-ink/wax printer, and reliable power supply to run the machines all the time.

Microfluidic thread-based analytical devices (μ TADs) have recently emerged as a possible solution to patterning problems since thin threads can conduct solutions in micro-volumes towards a target reaction zone. This is because threads are flexible hence can be manipulated in any form and direction without any problem, and with their small diameter, can wick and transport very small amounts of analyte (Li et al. 2010; Reches et al. 2010).

In this study, threads were used to create channels through which sample, conjugate reagents, and reaction complexes flow, thereby obviating the need for patterning necessary for multiplexed paper-based diagnostic devices. Since the membrane is an essential part for immobilisation of capture reagents, this design will allow the analyte-detector (conjugate) complex flowing through threads to be delivered to an underlying strip of nitrocellulose membrane containing immobilised capture antibodies for signal development.

2 Materials and methods

2.1 Materials

White polyester sewing thread (MH[®], Ningbo, China) and household sewing needle were purchased from local textile dealers. Mounting tape (Fantastick (FK-M485)[®], China) and food dyes (Three Parrots[®], L. Liladhar and Co., India) were purchased from local stationery shops and food markets, respectively. Nitrocellulose membrane, glass fibre conjugate pads, sample pads and absorbent pads were all supplied by Claremont Bio Solutions (CA, USA). Detection (conjugate) and capture antibodies, and antigens (Immunoglobulin G, Hepatitis B virus and *Helicobacter pylori*) were all supplied by Arista Biologicals (Allentown, PA, USA). Conjugate antibodies used were monoclonal mouse anti-*H. pylori* colloidal gold conjugate, clone 4 (CGHPY-0704), monoclonal mouse anti-HBsAg colloidal gold conjugate, clone 2 (CGHBS-0702), and goat anti-human IgG colloidal gold conjugate (CGIGG-0500). All conjugate antibodies were conjugated to a 40 nm colloidal gold particle at a concentration of 10 μ g/ml (OD540 = 10). Capture antibodies used were mouse anti-*H. pylori* antibody (ABHPY-0403), goat anti-HBsAg (ABHBS-0500) and goat anti-human IgG (ABIGG-0500). In vitro purified proteins from *H. pylori*, recombinant HBsAg strain produced in *pichia pastoris*, and human IgG purified by immunoaffinity from whole goat antisera, were used as antigens for *H. pylori*, Hepatitis B and IgG, respectively.

2.2 Design fabrication using food dyes

Red, yellow and green food dye solutions were prepared, and punched paper discs were immersed in the solutions and allowed to dry at room temperature. A pair of scissors was used to cut 2×10 mm strips of nitrocellulose membrane. Using an ordinary household sewing needle, a thread was sewn from the centre of the top side of a double-sided mounting tape to the lower side and through a dyed paper disc back to the top side of the tape further distal. This was done for the rest of the discs as depicted in Fig. 1a. A second layer of tape was applied to the bottom side of the tape to laminate the discs thereby protecting impregnated reagents from physical and chemical degradation. A plain paper disc was attached at the top of the sample inlet thread end at the centre to serve as sample pad. Nitrocellulose membrane strips were stuck in contact with thread ends emerging from below the tape, with thread ends overlapping the membrane strips by 1–2 mm, to facilitate transfer of solutions from threads to the membrane. At the distal ends of membrane strips, absorbent

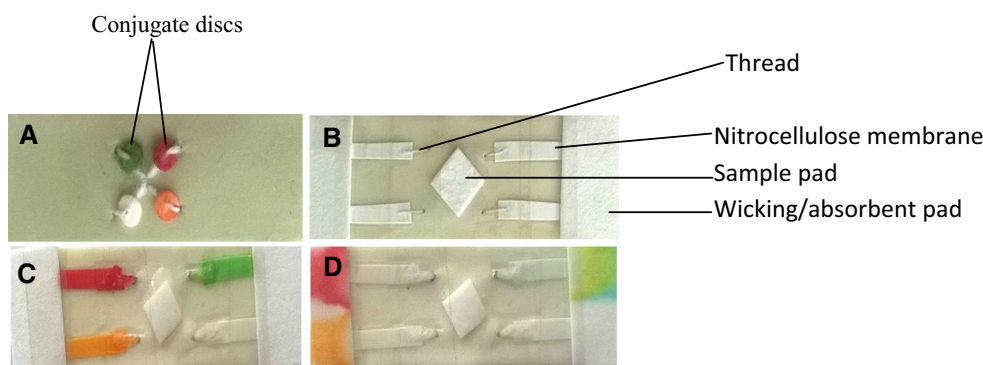


Fig. 1 Reagent flow through threads and nitrocellulose membrane. **a** Underside of tape indicating reagent-loaded paper discs (represented by red, green and orange dyes). **b** Top view of the device after assembly. **c** Flow of dyes from the middle layer of the tape through the

nitrocellulose membrane at the top after addition of water (sample). **d** Appearance of the membrane after all the dye has cleared out towards the absorbent pad

pads were attached to facilitate wicking and draining of excess solutions (Fig. 1b). To test the assembly, a few drops of water were added to the sample pad and observed for emergence of different dyes from under the tape via the thread onto the nitrocellulose membrane and absorbent pad further distal.

2.3 Immunochromatographic thread-based device

Glass fibre conjugate pad discs were each treated with 2.5 μ l of 10% BSA in PBS and allowed to dry at room temperature for 5 min and then at 50 °C for 10 min. Stock conjugate antibody (OD10) was diluted one-fifth in conjugate buffer (2% w/v BSA, 10% w/v sucrose and 0.1% v/v Tween 20 in Borate buffered saline). This was followed by addition of 5 μ l of gold conjugate antibody, each with a specific antibody type (anti-human IgG, anti-hepatitis B surface antigen and anti-*H. pylori*). The pads were dried at room temperature for 5 min and then at 50 °C for 10 min as above. Dried conjugate pads were kept in sealed bags with desiccant ready for assembly. Capture and control antibodies were diluted in PBS (pH 7.4) to a concentration of 0.5 mg/ml. 5% methanol was included to enhance fixation of antibodies to the membrane. Test and control lines were drawn on nitrocellulose membrane by using Lateral Flow Reagent Dispenser and Legato Syringe Pump (Claremont Bio Solutions, CA, USA). The dispense rate was 0.25 ml/min and voltage was set at 5v. After dispensing, the sheet was dried at 50 °C for 30 min and cut into 2 $\times$ 10 mm strips using a pair of scissors. This was followed by addition of 2 μ l of blocking buffer (0.1% v/v Tween 20, 5% w/v sucrose and 1% w/v BSA) per strip. After blocking, strips were dried as above and kept at room temperature in sealed bags with desiccant. This step was carried out for all capture antibodies (anti-human IgG, goat anti-Hepatitis B and mouse anti-*H. pylori*) and control antibody (Goat anti-mouse IgG).

3 Results

A four-arm design was demonstrated using food dyes, and flow of solutions was observed. This design combines both vertical and lateral flow across the layers of the device. The thread transported the solution vertically downwards from the sample pad at the top to the bottom of the tape. Then, there was a lateral movement through punched paper discs followed by a vertically upward movement of colour solution back to the top layer where lateral flow occurred along the nitrocellulose membrane and absorbent pad. The time interval between addition of water sample onto the sample disc and emergence of dye solution from under the tape was approximately 1–2 min.

Assembly of device for demonstration of reagent flow using red, green and orange food dyes was done as indicated in Fig. 1a–d. After addition of water drops at the sample pad (middle), food dyes on paper discs dissolved to subsequently flow to the top onto the nitrocellulose membrane. The membranes were clear of dyes within 3–5 min (Fig. 1d). This corresponds to the time required to drain excess sample and reagents from the membrane, so the signal can be visualised.

After demonstration with food dyes, the device was tested using antibodies to mimic actual test design for infectious disease rapid diagnostics. Colloidal gold-labelled detection antibodies were dried on glass fibre to make conjugate pads (used in place of food dye discs). With the capture antibodies immobilised on the NC membrane, introducing a sample at the top disc initiated vertical and lateral flow of reagent complexes that completed the test. A red line on the nitrocellulose membrane at a position where capture antibodies were immobilised indicated *positive* test, while a visible control line represented a *valid* test. Figure 2 represents a duo device for simultaneous detection of a bacterial and viral target antigen. *H. pylori* and Hepatitis B represented here can be substituted by any other target depending on the need and

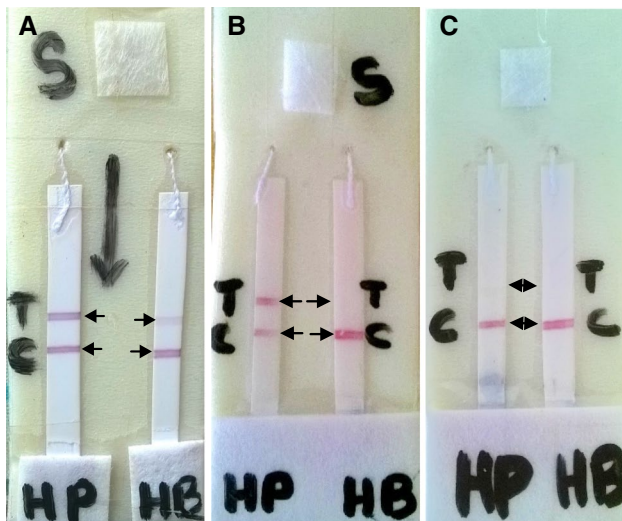


Fig. 2 Duo-test device. Note that test A is positive for both *H. pylori* (HP) and Hepatitis B surface antigen (HB), test B is positive for *H. pylori* (HP) and negative for Hepatitis B (HB) while test B is negative for both HP and HB

circumstances. The duo test signals began to show within the first 5 min after addition of 30–40 μl of sample mixture, and was ready for reading within 10 min. Positive and negative tests were clearly visible as described under Fig. 2a–c.

The lowest detectable antigen concentration for *H. pylori* on naked strips was 300 ng/ml with 30 ng/ml indicating a faint signal (Fig. 3a). For the case of Hepatitis B, the limit of detection for single strips was 80 $\mu\text{g}/\text{ml}$ (Fig. 3b). On assembled devices, estimated limit of detection was observed to be close to the above (below 100 and 400 ng/ml for *H. pylori* and Hepatitis B, respectively), with signal intensity being slightly lower (Fig. 3c).

The signal for multiplex design was read between 5 and 10 min and results are as presented in Figs. 4 and 5. Time for development of test results was comparable to what is usually observed in all commercial lateral flow strip tests. Also, there was no notable difference in time required by a duo test (2 strips) or multiplex test (6 strips), although slightly higher sample volume was required in case of multi-strip test ranging between 30 and 50 μl per multiplex run while less than 30 μl was required by single or double strip tests.

4 Discussion

This study demonstrates the feasibility of combining hydrophilic threads with other components of lateral flow tests to create a multiplex device that can potentially be used at point-of-care. Fabrication and assembly of this device proceeded in a three-dimensional format common for microfluidic analytical devices (Martinez et al. 2008), with the thread forming a channel that can cross multiple layers back and forth. The food dyes employed in these experiments reflected the flow of sample and reagents during actual testing. The discs were made of glass fibre using ordinary office punching machine. Polyester sewing thread used was hydrophilic, therefore requiring no prior treatment to improve fluid wicking. It took approximately 1 min for the dyes to emerge from under the tape. The dyes were visible as they wicked along the thread and membrane. The time taken by the dyes to clear from the membrane strips (approximately 5–10 min) could be equated to the time required to clear excess sample and reagents from the nitrocellulose membrane to allow for the signal to be clearly visible. Discs embedded between layers of mounting tape carry reagents that are necessary for the test. It is expected that these layers of tape help in protection

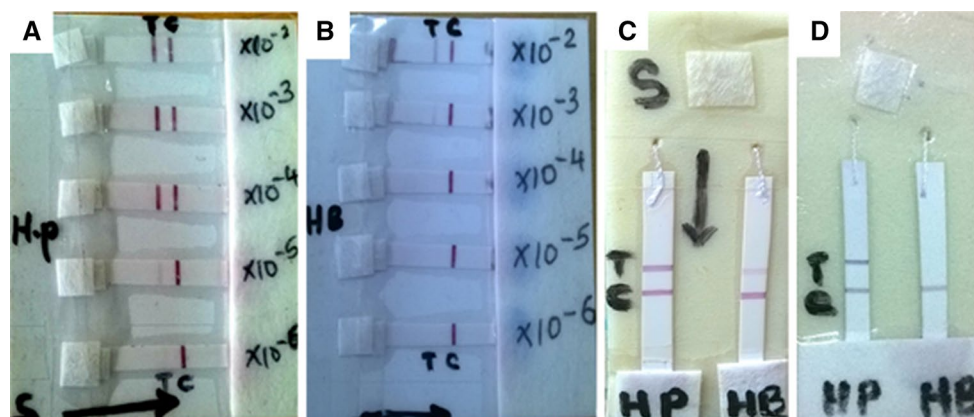


Fig. 3 Detection limit for *H. pylori* and Hepatitis B strips and duo device. Tested concentrations on strips in **a** range from 30 $\mu\text{g}/\text{ml}$ to 3 ng/ml while strips in **b** range from 80 $\mu\text{g}/\text{ml}$ to 8 ng/ml. Tested con-

centrations on the dual device (**c, d**) were 100 and 400 ng/ml for *H. pylori* and Hepatitis B, respectively

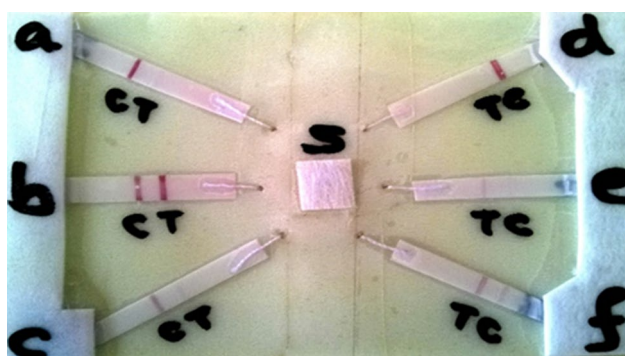


Fig. 4 Multichannel test device with interpretation of possible test results. Negative results (a, d); positive results (b, f); doubtful (c); invalid (e); C control, T test

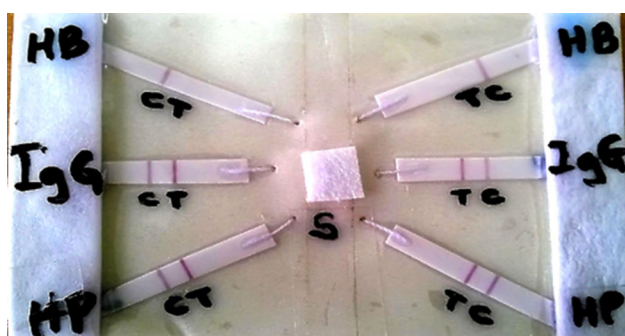


Fig. 5 Six-channel multiplex device. Note that the test is positive for IgG and *H. pylori* (HP) but negative for Hepatitis B (HB). T test, C control

of the reagent-loaded discs thereby increasing their shelf life (Fenton et al. 2009).

Using the above design, a device was fabricated for detection of antigens in solution. Detection antibodies conjugated to gold nanoparticles were dried on glass fibre conjugate pad. On a nitrocellulose membrane, capture antibodies were immobilised. The conjugate pads were laminated between layers of mounting tape and connected to the sample pad and nitrocellulose membrane above via hydrophilic threads. The double-sided mounting tape served as an adhesive backing for attachment of the sample pad and nitrocellulose membrane. It also served as laminate for the conjugate pads, as well as provide mechanical strength to the device. Under resource-constrained settings, this tape could be used as substitute for hard cassettes and other forms of housing (Fenton et al. 2009).

The designs fabricated and tested using antibodies for detection of antigens (*H. pylori*, Hepatitis B and IgG) demonstrate the potential of applying immunochromatographic thread-based platforms in diagnosis of infectious diseases. In previously described immunochromatographic assays on thread (ICAT), threads were used as both wicking (transport)

and reaction surfaces (Zhou et al. 2012). Both the conjugate/detection and capture antibodies were immobilised on threads or yarns. In the current design, threads are only used to facilitate wicking of solution along the test platform as detection antibodies are stored on glass fibre and capture antibodies immobilised on nitrocellulose membrane just like on typical lateral flow strip tests. With this design, only the thread is introduced as a new component, other components such as sample loading pad, conjugate pad, nitrocellulose membrane and wicking pad remain the same.

Multiplexed platforms were made easy due to the flexibility and durability of threads (Ballerini et al. 2011). A desired number of channels were created using a sewing needle. Using threads like in the current platform facilitates multiplexing since threads can be modified to form complex networks without the need for complicated patterning. In paper-based microfluidic analytical devices, patterning of paper by various approaches such as wax-printing, laser cutting, photolithography, etching and plotting is essential for multiplexing (Carrilho et al. 2009; Lu et al. 2009; Fenton et al. 2009; Martinez et al. 2008; Abe et al. 2008; Bruzewicz et al. 2008). Using threads obviates the need for further patterning making this approach a promising alternative for designing low-cost multiplexed POC diagnostic devices. Hydrophilic cotton, polyester, acrylic and other types of thread materials are all suitable and can be used for this purpose although there may be variations in the flow dynamics depending on the type of fibres with which the threads are made (Nilg-haz et al. 2014). If threads are hydrophobic they are usually treated using hydrophilising agents before they can be used. Since only simple tools such as sewing needles, threads and tape are needed during fabrication process, these platforms can simply be adopted as potential diagnostics manufacturing alternative for the developing world.

To run a six-channel device, a sample volume of approximately 30–50 μ l was required. This volume is expectedly higher than what is normally required for a single test, e.g. between 10 and 20 μ l for malaria RDT (Foster et al. 2014) because in multiplexed designs, the sample must be distributed along multiple channels. However, this volume is comparable to what has been reported for numerous other lateral flow devices where the sample in the range of 15–200 μ l has been reported (Sharma et al. 2015). Threads used in this design are thin (approximately 200–300 μ m in diameter), resulting in minimal fluid/sample retention during testing. Despite this fact, the flow rate was not compromised as the sample travelled across the membrane to the absorbent pad within 3 min, and signal started forming within the first 5 min and the test was ready within 15 min. This too, is comparable to most lateral flow devices reported previously, including single strip tests (Posthuma-Trumpie et al. 2009).

The lowest detection limit was found to be within the range of 30–300 ng/ml, which is comparable to or vary

slightly from other lateral flow tests described elsewhere (Gessler et al. 2007; Jimenez et al. 2017), although the limit was higher for Hepatitis B surface antigen. The dynamic range for these devices may sometimes fall in the pico range implying very high sensitivity. Reduced sensitivity may be a result of many factors, including biological state of conjugate and capture antibodies, device assembly and storage conditions for reagents and assembled devices. Laminating the section of NC membrane with a transparent adhesive tape caused fast flow of the reaction mixture, reducing the time required to complete the test. However, it also reduced the sensitivity of the test either by decreasing the intensity of the test line or failure to develop the test line at normally detectable analyte levels. This could probably be attributed to the fast flow rate that reduces contact time between the analyte-detector antibody complex and the capture antibody at the test and control lines. Lamination offers protection to the membrane against physical damages like scratching or wetting. Therefore, alternative protective measures that do not affect signal strength or sensitivity should be considered.

In this study, testing was done using purified antigens diluted in wash buffer (0.05% Tween 20 in PBS). Body fluids resembling this medium are such as serum, plasma, saliva and urine. However, challenges in flow dynamics, reaction time and membrane clearance time are expected when a sample with different colorimetric and viscosity properties such as blood, stool or pus is used. Evaluation of these sample media and associated flow parameters will be performed at a later stage.

Targets for this study were of diverse nature: immunoglobulins, bacterial antigen and viral antigens, a combination that minimises the likelihood of cross reactivity between the antigens. This selection was intended to demonstrate that multiplex POC diagnostic devices can be designed to detect pathogens that fall into diverse taxonomic groups. This implies that if carefully designed it can incorporate viral, bacterial, protozoal and fungal pathogens on one multiplex device for enhanced throughput and diversity. This is a promising prospect for addressing the challenge of diagnosing non-malarial febrile illnesses (NMFI), whose aetiology has been reported to be significantly diverse, comprising of bacterial, viral, fungal, protozoal and other causes (Kiemde et al. 2016; Acestor et al. 2012; Bisoffi and Buonfrate 2013; Okiro and Snow 2010; Mahende et al. 2014). To enable accurate and timely diagnosis of such complex clinical presentation, multiplexed diagnostic platforms will be explored in the future.

During subsequent steps towards development of a prototype based on this platform, there are important issues that will need to be taken into consideration including: choice of thread type, standardisation of procedure for reagents/biologicals preparation and device fabrication, standardisation of assay time as well as sample size determination. Each of these

is significant during evaluation of a prototype diagnostic test as they may singly or collectively affect the sensitivity, and/or specificity of the test.

Most of the items used for fabrication of this device such as threads, needle, adhesive tape and papers are easily available and cheap. Conjugate and capture antibodies dried on the glass fibre and nitrocellulose membranes can remain stable for months. To run the test, the operator only applies the sample on the sample pad and observes for only a few min to read the results. These attributes will result in simple devices that are affordable, user-friendly, robust, equipment free, and deliverable to end users. This platform has great potential towards development of low-cost multiplex diagnostic tests for simultaneous detection of aetiologies for neonatal and maternal septicemia as well as non-malarial febrile illnesses, which are of significant importance in resource-limited settings.

5 Conclusion

Multiplexed immunochromatographic diagnostic platforms can be designed using hydrophilic threads as fluid distribution channels. Using threads obviates the need for patterning of paper that would otherwise require additional equipment like solid-ink (wax) printer or carbon dioxide laser cutter. Combining glass fibre, thread, nitrocellulose membrane and mounting tape produces a firm device that can be used at the point-of-care. More work on these platforms is needed to transform the concept into practical diagnostic solution to facilitate manufacture of low-cost devices for diagnosis of multiple illnesses at the point-of care in resource-limited settings.

Acknowledgements We thank the technical staff at NM-AIST for their cooperation and support during execution of this work. This work was supported by the Tanzania Commission for Science and Technology (COSTECH) through the Nelson Mandela African Institution of Sciences and Technology (NM-AIST) Graduate Scholarship.

Authors' contributions MS and JB developed the research plan. MS and DM carried out research work with the help of JB. MS interpreted the data and prepared the manuscript. DM and JB edited and reviewed the manuscript. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

References

- Abe K, Suzuki K, Citterio D (2008) Inkjet-printed microfluidic multi-analyte chemical sensing paper. *Anal Chem* 80:6928–6934
- Acestor N, Cooksey R, Newton PN et al (2012) Mapping the aetiology of non-malarial febrile illness in Southeast Asia through a

- systematic review-terra incognita impairing treatment policies. *PLoS ONE* 7:e44269
- Ballerini DR, Li X, Shen W (2011) Flow control concepts for thread-based microfluidic devices. *Biomicrofluidics* 5:014105. <https://doi.org/10.1063/1.3567094>
- Bisoffi Z, Buonfrate D (2013) When fever is not malaria. *Lancet Glob Health* 1:e11–e12
- Boisen ML, Oottamasathien D, Jones AB, Millett MM, Nelson DS, Bornholdt ZA et al (2015) Development of prototype filovirus recombinant antigen immunoassays. *J Infect Dis* 212(Suppl. 2):S359–S367
- Bruzewicz DA, Reches M, Whitesides GM (2008) Low-cost printing of poly (dimethylsiloxane) barriers to define microchannels in paper. *Anal Chem* 80:3387–3392
- Carrilho E, Martinez AW, Whitesides GM (2009) Understanding wax printing: a simple micropatterning process for paper-based microfluidics. *Anal Chem* 81:7091–7095
- Fenton EM, Mascarenas MR, Lopez GP, Sibbett SS (2009) Multiplex lateral-flow test strips fabricated by two-dimensional shaping. *ACS Appl Mater Interfaces* 1:124–129
- Foster D, Cox-Singh J, Mohamad DSA, Krishna S, Chin PP, Singh B (2014) Evaluation of three rapid diagnostic tests for the detection of human infections with *Plasmodium knowlesi*. *Malar J* 201413:60. <https://doi.org/10.1186/1475-2875-13-60>
- Gessler F, Pagel-Wiederb S, Avondet M, Bfhnela H (2007) Evaluation of lateral flow assays for the detection of botulinum neurotoxin type A and their application in laboratory diagnosis of botulism. *Diagn Microbiol Infect Dis* 57:243–249
- Hossain SMZ, Ozimok C, Sicard C, Aguirre SD, Ali MM, Li YF, Brennan JD (2012) Multiplexed paper test strip for quantitative bacterial detection. *Anal Bioanal Chem* 403(6):1567–1576
- Jimenez A, Rees-Channer RR, Perera R, Gamboa D, Chiodini PL, González IJ, Mayor A, Ding XC (2017) Analytical sensitivity of current best-in-class malaria rapid diagnostic tests. *Malar J* 16:128. <https://doi.org/10.1186/s12936-017-1780-5>
- Kamphee H, Chaiprasert A, Prammananan T, Wiriyachaiyorn N, Kanchanavee A, Dharakul T (2015) Rapid molecular detection of multidrug-resistant tuberculosis by PCR-nucleic acid lateral flow immunoassay. *PLoS ONE* 10:e0137791
- Kiemde F, Spijker R, Mens PF, Tinto H, Boele M, Schallig HDFH (2016) Etiologies of non-malaria febrile episodes in children under 5 years in sub-Saharan Africa. *Trop Med Int Health* 21(8):943–955
- Law JWF, Ab Mutalib NS, Chan KG, Lee LH (2014) Rapid methods for the detection of foodborne bacterial pathogens: principles, applications, advantages and limitations. *Front Microbiol* 5:770
- Li X, Tian J, Shen W (2010) Thread as a versatile material for low-cost microfluidic diagnostics. *ACS Appl Mater Interfaces* 2(1):1
- Lu Y, Shi W, Jiang L, Qin J, Lin B (2009) Rapid prototyping of paper-based microfluidics with wax for low-cost, portable bioassay. *Electrophoresis* 30:1497–1500
- Mahende C, Ngasala B, Lusingu J et al (2014) Aetiology of acute febrile episodes in children attending Korogwe District Hospital in north-eastern Tanzania. *PLoS ONE* 9:e104197
- Martinez AW, Phillips ST, Butte MJ, Whitesides GM (2007) Patterned paper as a platform for inexpensive, low volume, portable bioassays. *Angew Chem Int Ed Engl* 46(8):1318–1320. <https://doi.org/10.1002/anie.200603817>
- Martinez AW, Phillips ST, Whitesides GM (2008) Three-dimensional microfluidic devices fabricated in layered paper and tape. *PNAS* 105(50):19563–19564. <https://doi.org/10.1073/iti05008105>
- Matsuura K, Chen K, Tsai C, Li W, Asano Y, Naruse K, Cheng C (2014) Paper-based diagnostic devices for evaluating the quality of human sperm. *Microfluid Nanofluid* 14(16):857–867
- May K (1991) Home tests to monitor fertility. *Am J Obstet Gynecol* 165:2000–2002
- Nielsen K, Yu WL, Kelly L, Bermudez R, Renteria T, Dajer A et al (2008) Development of a lateral flow assay for rapid detection of bovine antibody to *Anaplasma marginale*. *J Immunoass Immunochem* 29:10–18
- Nilghaz A, Zhang L, Li M, Ballerini DR, Shen W (2014) Understanding thread properties for red blood cell antigen assays: weak ABO blood typing. *ACS Appl Mater Interfaces* 6:22209–22215
- Okiro EA, Snow RW (2010) The relationship between reported fever and *Plasmodium falciparum* infection in African children. *Malar J* 9:99
- Peeling RW, Holmes KK, Mabey D, Ronald A (2006) Rapid tests for sexually transmitted infections (STIs): the way forward. *Sex Transm Infect* 82(Suppl 5):v1–v6
- Posthuma-Trumpie GA, Korf J, van Amerongen AA (2009) Lateral flow (immuno) assay: its strengths, weaknesses, opportunities and threats. A literature survey. *Anal Bioanal Chem* 393:569–582
- Reches M, Mirica KA, Dasgupta R, Dickey MD, Butte MJ, Whitesides GM (2010) Thread as a matrix for biomedical assays. *ACS Appl Mater Interfaces* 2(6):1722
- Rohrman BA, Leautaud V, Molyneux E, Richards-Kortum RR (2012) A lateral flow assay for quantitative detection of amplified HIV-1 RNA. *PLoS ONE* 7:e45611
- Shankar BP, Manjunatha Prabhu BH, Chandan S, Ranjith D, Shivakumar V (2010) Rapid methods for detection of veterinary drug residues in meat. *Vet World* 3(5):241–246
- Sharma S, Zapatero-Rodríguez J, Estrela P, O’Kennedy R (2015) Point-of-care diagnostics in low resource settings: present status and future role of microfluidics. *Biosensors* 5:577–601. <https://doi.org/10.3390/bios5030577>
- Shyu RH, Shyu HF, Liu HW, Tang SS (2002) Colloidal gold-based immunochromatographic assay for detection of ricin. *Toxicol* 40:255–258. [https://doi.org/10.1016/S0041-0101\(01\)00193-3](https://doi.org/10.1016/S0041-0101(01)00193-3)
- Sicard C, Gien C, Aubie B, Wallace D, Jahanshahi-Anbuhi S, Pennings K, Daigger GT, Pelton R, Brennan JD, Fillipe CD (2015) Tools for water quality monitoring and mapping using paper-based sensors and cell phones. *Water Res* 2015(70):360–369. <https://doi.org/10.1016/j.watres.2014.12.005>
- Toldra F, Reig M (2006) Methods for rapid detection of chemical and veterinary drug residues in animal foods. *Trends Food Sci Technol* 17:482–489
- Urdea M, Penny LA, Olmsted SS, Giovanni MY, Kaspar P, Shepherd A, Wilson P, Dahl CA, Buchsbaum S, Moeller G, Burgess DCH (2006) Requirements for high impact diagnostics in the developing world. *Nature* 444(Suppl. 1):73–79
- Weng X, Gaur G, Neethirajan S (2016) Rapid detection of food allergens by microfluidics ELISA-based optical sensor. *Biosensors* 6:24. <https://doi.org/10.3390/bios6020024>
- Zasada AA, Formińska K, Zacharczuk K, Jacob D, Grunow R (2015) Comparison of eleven commercially available rapid tests for detection of *Bacillus anthracis*, *Francisella tularensis* and *Yersinia pestis*. *Lett Appl Microbiol* 60:409–413. <https://doi.org/10.1111/lam.12392>
- Zhou G, Mao X, Juncker D (2012) Immunochromatographic assay on thread. *Anal Chem*. <https://doi.org/10.1021/ac301082d>