International Journal of Research in Medical Sciences
*Seth M et al. Int J Res Med Sci. 2017 Nov;5(11):5052-5059*
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012

DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20174969

**Original Research Article**

# Multiplex paper-based designs for point-of-care (POC) diagnostics

**Misago Seth*, Joram Buza**

Department of Global Health and Biomedical Sciences, Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania

**Received:** 16 June 2017
**Accepted:** 18 July 2017

***Correspondence:**
Dr. Misago Seth,
E-mail: sethm@nm-aist.ac.tz

**Copyright:** © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

## ABSTRACT

**Background:** Accurate and timely diagnosis is usually the first step towards appropriate disease management. In resource-limited settings, healthcare workers lack proper facilities to perform vital tests, and the diagnosis of disease is often determined by non-specific, physiological symptoms alone. Simple and rapid tests are needed as prerequisite tools for patient care and must conform to the criteria set out by the World Health Organization. To address the challenge of specific diagnosis for diseases that present with similar symptoms, multiplex diagnostic platforms must be designed.
**Methods:** Simple designs for multiplexed paper-based diagnostic platforms were developed. Laser cutting and wax printing were used to create specific patterns on paper to guide the flow of sample and reagents towards reaction zones. Multi-arm and single strip multiplex platforms were designed and tested using Human Immunoglobulin G, Hepatitis B virus surface antigen (HBsAg), and *Helicobacter pylori* antigens.
**Results:** All designs produced promising results with the lowest limit of detection for antigens being 30ng/ml for single strip designs.
**Conclusions:** The use of glass fibre conjugate pad was found to be more sensitive compared wax-printed chromatography paper. Results from this study indicate great potential for further application in development of diagnostic low-cost paper-based diagnostic devices.

**Keywords:** Assured diagnostics, low-cost diagnostics, Multiplex devices, paper-based diagnostic platforms, Point-of-care diagnostics

## INTRODUCTION

Appropriate patient care or disease management is highly dependent on timely and accurate diagnosis, which is insufficiently available in resource-limited settings, culminating into symptomatic diagnosis and treatment most of the time.[1,2] Simple and rapid tests are needed as prerequisite tools for patient care and must conform to the 'ASSURED' criteria set out by the World Health Organization (WHO). An ideal test must be Affordable by those at risk of infection, Sensitive (few false-negative results), Specific (few false-positive results), User-friendly (simple to perform by persons with little training), Rapid/robust, Equipment-free and Deliverable to those who need it.[3-5]

In recent years, paper has emerged as an important substrate for fabrication of low-cost, simple diagnostics for use in limited-resource settings.[6-8] Desirable features of paper include its ubiquitous abundance, mechanical strength, and flexibility. Paper can wick and distribute aqueous fluids, and has been in use for a long time as an analytical platform. Also, paper can be modified to incorporate functional groups, can easily be transported and stored, is compatible with most printing technologies, and can easily be disposed of incineration.[9,10] Cellulose-

based materials such as chromatography and filter papers have found wide applications in making dipsticks and microfluidic paper-based analytical devices.[11] while nitrocellulose membranes are mostly used in Lateral flow immunoassays.[12]

There are cases where multiple diseases or conditions need to be diagnosed together or differentiated, especially if observable symptoms are similar. The best example is the case of febrile illnesses in malaria-endemic regions.[13-17] Patients predominantly present with fever symptoms and cannot be accurately diagnosed without a specific test. When the most common etiology of fever (malaria) is ruled out, identifying the exact cause of fever is usually done clinically in most cases, or if resources allow, through sequential testing of the likely fever etiologies to reach definitive diagnosis.[18]

Designing multiplexed test devices is therefore very important for enhancing timely diagnosis as well as minimizing the cost that would otherwise be incurred if sequential testing was to be performed.[17] Using paper, various patterning methods have been described and tested in previous studies. These methods include cutting, wax printing, photolithography, plotting, ink etching, and plasma etching, among others.[7,19-24] Apart from clinical applications, other point-of-need applications for multiplex paper-based devices may include their use in qualitative/semi-quantitative detection of antimicrobial residues in milk and other foods of animal origin where other testing methods are currently being applied, and screening for environmental contamination with heavy metals.[25-28] Of all the above applications, the use of these devices for point-of-care diagnosis at health outlets to facilitate patient care in the developing world remains the most compelling. In this article, we describe simple designs for multiplexed paper-based diagnostics where a combination of laser-cutting and wax-printing was used to create channels and barriers to direct the flow of sample and reagents on paper. The designs were tested using Human Immunoglobulin G, Hepatitis B virus surface antigen (HBsAg), and *Helicobacter pylori*.

## METHODS

### *Antibodies and antigens*

Antibodies and antigens used in this study were obtained from 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 40nm colloidal gold particle at a concentration of 10µg/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). Invitro 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.

The device platforms were designed using glass fiber pads, chromatography paper and nitrocellulose membrane. Chromatography paper and fiberglass were used as sample and conjugate pads while nitrocellulose was used as capture membrane. Three different designs are described in this paper.

### *Multi-channel multiplex designs*

In the first design, conjugate pad and nitrocellulose membrane were both cut using laser cutter per the plots made on a computer using Corel 7 drawing software (Figure 1). In the other design, a sketch drawn using computer program were printed on chromatography paper using Xerox 7560 solid ink (wax) printer to create hydrophobic barriers and hydrophilic chambers and channels for deposition and flow of conjugate reagents and sample (Figure 1). Printed paper was heated in hot-air oven for 30 seconds at 150$^{o}$C with the printed side facing up to melt the wax through the paper thereby creating continuous channels and barriers. The designs were tested by assembling a device for simultaneous detection of Helicobacter pylori (bacteria), Hepatitis B viral antigen S (virus), and Human Immunoglobulin G. Each arm of the conjugate pad was treated with 2.5µl of 10% BSA in PBS and allowed to dry at room temperature for 5 minutes and then at 50 $^{0}$C for 10 minutes. 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 5µl of gold conjugate antibody to the conjugate pad, each arm with a specific antibody type. The pads were dried at room temperature for 5 minutes and then at 50 $^{0}$C for 10 minutes as above. Dried conjugate pads were kept in sealed bags with desiccant ready for device assembly.

The nitrocellulose (capture) membranes appropriately cut to fit the design were treated with the specific capture antibodies. Capture antibodies were diluted in PBS (pH 7.4) to a concentration of 0.5mg/ml, with 5% methanol included to enhance fixation of antibodies to the membrane. A total of 3µl were applied to the membrane in small quantities (0.5µl per round). The membrane was dried at room temperature for 2 minutes followed by 10 minutes at 50$^{0}$C. This was followed by addition of 2 µl of blocking buffer (0.1% v/v Tween 20, 5% w/v sucrose and 1% w/v BSA) per arm, and then dried as above.

Assembly of the multichannel device was accomplished by first sticking the nitrocellulose membrane on an adhesive tape/plastic backing card placed on a flat surface. At the distal end of each arm, appropriately cut absorbent filter papers were placed on top of the membrane in an overlapping fashion to serve as wicking

or absorbent pads. This was followed by overlaying the conjugate (or conjugate cum sample) pad at the center position on top of the membrane. For the first design (Figure 3A), a sample pad was placed on top of the fiberglass conjugate pad to complete the device assembly. Assembled devices were tested using a serially diluted mixture of target antigens to establish the limits of detection of each against respective antigens.

### *Single channel multiplex design*

The aim was to assess the possibility of combining tests for various analytes on a single strip, comprised of a single conjugate pad and capture (nitrocellulose) membrane. In this design, gold-conjugated antibodies specific to target antigens Human Immunoglobulin G, Hepatitis B, and *H. pylori* (all from Arista Biologicals, Allentown, PA, USA) were blotted on a single piece of glass fiber pad either individually or as pooled solution. In the first approach 5µl of each gold conjugate was applied on the pad, followed by the next until all were added. The pad was dried between additions, first at room temperature for 5 minutes, followed by 2 minutes at 50ºC in an oven. After the final addition, the conjugate pad was dried for a further 10 minutes at 50ºC and kept dry until use. Alternatively, all conjugates were mixed together to make one pooled solution. About 10-15 µl of the conjugate mixture was applied to the conjugate pad and dried as above. Prior to addition of conjugates, the glass fiber pad was pre-treated with 5µl of 10% BSA and dried as described above. Capture antibodies were diluted in PBS (pH 7.4) and 5% methanol to a working concentration of 0.5mg/ml. A total of 3µl of each antigen-specific capture antibody were applied in small quantities (0.5µl per round) at specifically identified test zones on the membrane. The membrane was dried at room temperature for 2 minutes followed by 10 minutes at 50$^0$C. The membrane was blotted with 2 µl of blocking buffer (0.1% v/v Tween 20, 5% w/v sucrose and 1% w/v BSA) and dried as above. The two methods of conjugate pad treatment were compared by running a sample of antigen mixture at the concentration of 30µg/ml and response was observed within 10 minutes after sample addition.

## RESULTS

### *Determining optimal conjugate dilution*

To determine optimal dilution for conjugate, stock conjugates with optical density (OD) of 10 at 540nm wavelength were diluted serially and then applied to conjugate pads. The concentration of capture antibodies on nitrocellulose membrane was fixed at 0.5mg/ml, and 3µl of each antibody were applied in several rounds of small quantities (0.5µl) at test zones. The pads and membranes were dried at room temperature for 5 minutes, then at 50$^0$C for 10 minutes. Stock antigens were diluted to a mixture concentration of 30µg/ml and applied to the device after assembly. The response was as shown below (Figure 4).

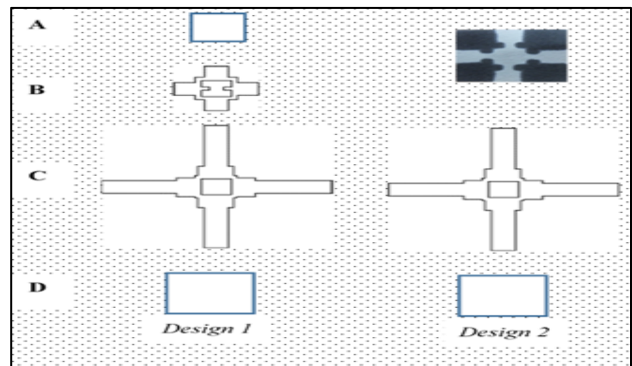



**Figure 1: Components of four-channel multiplex designs (laser-cut and wax-printed). Sample pad (a), conjugate pad (b), lateral flow nitrocellulose membrane (c), and absorbent pad (d). Note that for design 2, both the sample and conjugate pads are printed with wax on same paper.**

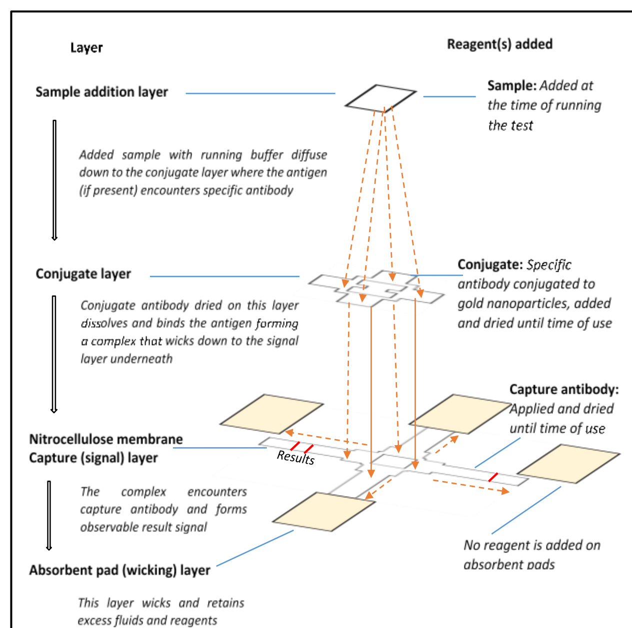



**Figure 2: Schematic representation of different layers (exploded view) of the multiplex device showing flow of reagents and fluids between layers when a test is performed.**

### *Using fiberglass conjugate pad*

Each of the three positive arms showed response at various level of conjugate dilution. Reaction signal was clear up to the 1/8th dilution of the conjugates (Figure 4C) but signal strength decreased upon further dilution. The highest working conjugate dilution was found to be 8-fold.

***Using chromatography paper as both sample and conjugate pad***

From the above image panel (Figure 5), signal can be observed up to the 1/4th dilution of the conjugates (Figure 5B). The conjugate part was found to still contain some residual conjugate even after sample and buffer wash. Overall, it appears that the glass fiber conjugate pads offer complete conjugate release compared to chromatography paper, hence could be a better medium for multiplex rapid diagnostic devices.

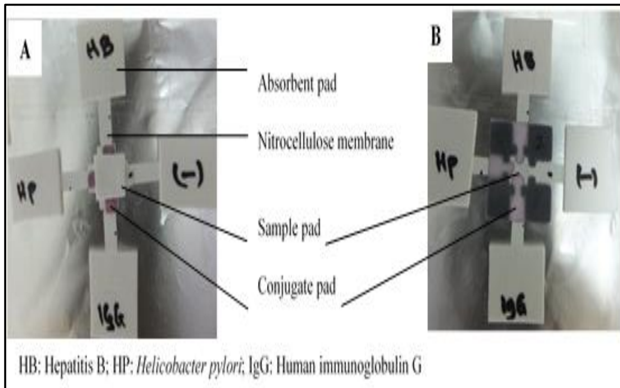
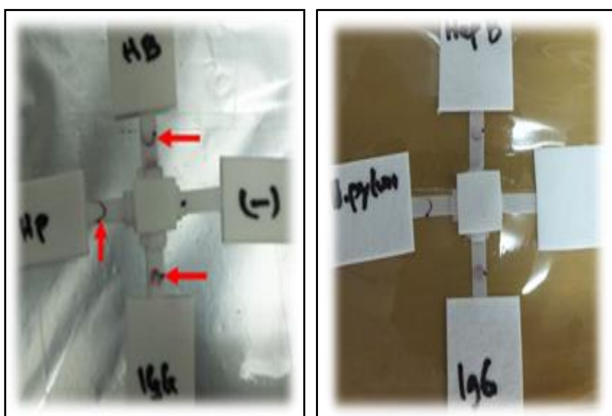


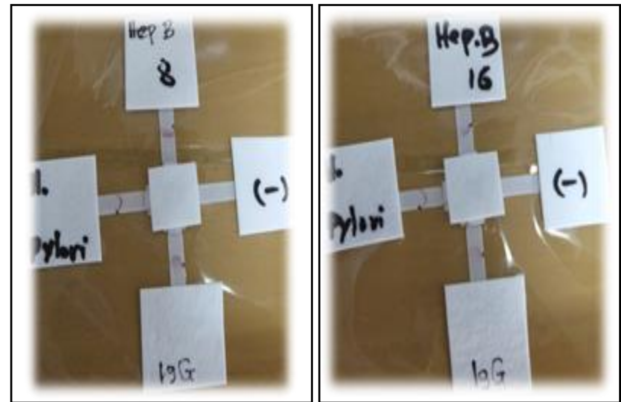

**Figure 3: Device assembly before sample application. A) Radial design with glass fiber conjugate pad and separate sample pad cut from Whatman filter paper. B) Conjugate and sample pads printed on chromatography paper.**

***Determining the limit of detection***

Antigens were mixed together and serially diluted to determine the limit of detection of the device. Serial dilution followed a hundred-fold initial dilution of stock antigens to approximately 300µg/ml followed by ten-fold dilutions up to 30ng/ml. Antigen mixture at each level of concentration was used to test the devices. Results are presented in the panel below (Figures 6 and 7).




A 1/2x dilution B 1/4x dilution




C 1/8x dilution D 1/16x dilution

**Figure 4: Determining optimal conjugate dilution for use on glass fiber conjugate pads. Note the decrease in signal intensity from A to D.**

Using glass fibre conjugate pad,

Using Chromatography paper conjugate.

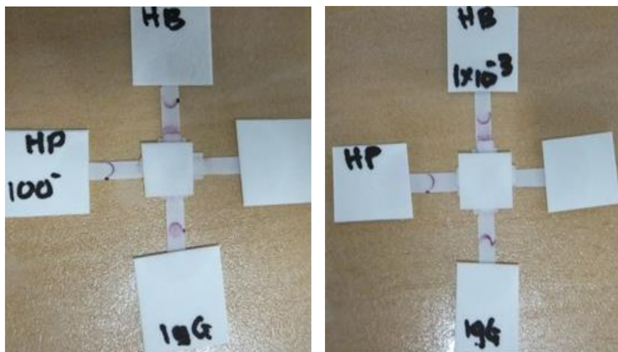
Test signal was observable for all three analytes up to the antigen concentration of 300ng/ml when glass fibre conjugate pad was used. In this case, only IgG showed positive signal at lower antigen concentration of 30ng/ml (Figure 6D). On devices made using chromatography paper as sample and conjugate pads, sensitivity was lower as the antigen limit of detection was found to be 3.0µg/ml except for IgG which was detected even at 30ng/ml (Figure 7D). Based on these results, the average limit of detection could be around 300ng/ml as shown in Figure 6. Only IgG showed response at lower concentrations.

A 1/2x dilution B 1/4x dilution C 1/8x dilution

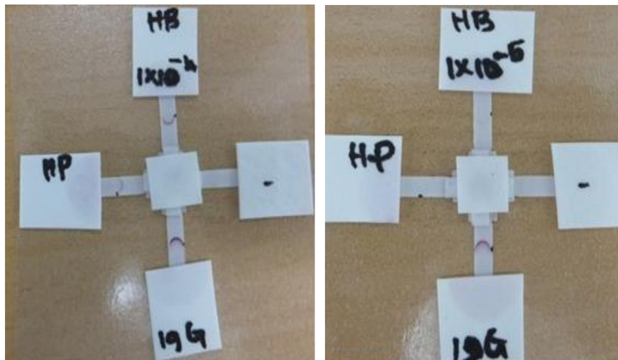
**Figure 5: Determining optimal conjugate dilution for use on wax-printed chromatography paper conjugate chambers. The signal intensity decreases with increasing conjugate dilution, only visible up to 4x dilution.**

***Conjugate optimization and limit of detection for single strip design***

For single strip test, evaluation of optimal method for conjugate application revealed no difference in signal intensity or time taken for signal to develop, whether conjugates are pooled or added one-by-one onto the conjugate pad (Figure 8A). Optimal conjugate dilution in this case was found to be 1/4th of the original (OD540=10) stock (Figure 8B). Positive signal was observed shorter than five minutes since sample addition. Signal was clear at antigen concentration 300ng/ml and above, although much lower antigen concentration (30 ng/ml) still produced visible signal (Figure 9).




A 1/100x dilution (30µg/ml) B 1/10-3x dilution(3.0µg/ml)

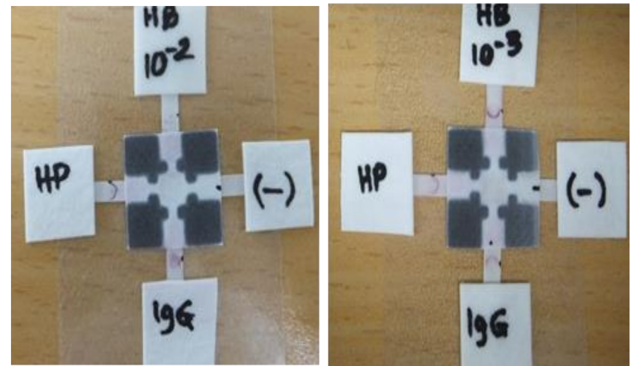



C 1/10$^{-4}$x dilution (300ng/ml) D 1/10$^{-5}$x dilution (30ng/ml)

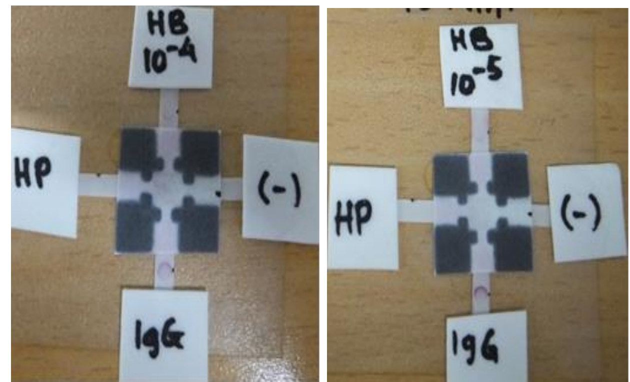
**Figure 6: Limit of detection for devices employing glass fiber conjugate pads.**

## DISCUSSION

Simple designs for multiplexed lateral flow immunochromatographic tests were tested in this study. Materials used have already been described and tested previously for similar purposes and results were promising.[2,3,9,10,29] Different designs were compared with respect to their potential as diagnostic prototypes. The study has demonstrated the possibility of using paper devices to simultaneously diagnose or detect multiple analytes in fluids. These may be applied in detection of disease-causing pathogens in people seeking healthcare at health facilities especially in developing countries where resources for advanced diagnosis may be limited.[5]

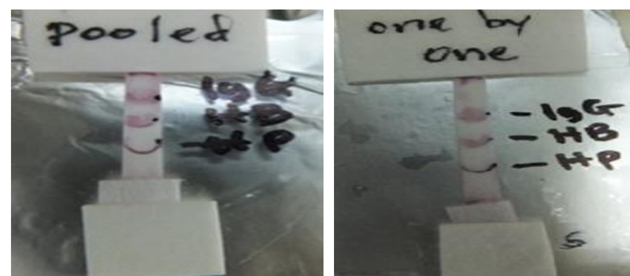



A: 1/100x dilution (30µg/ml) B 1/10-3x dilution (3.0µg/ml)

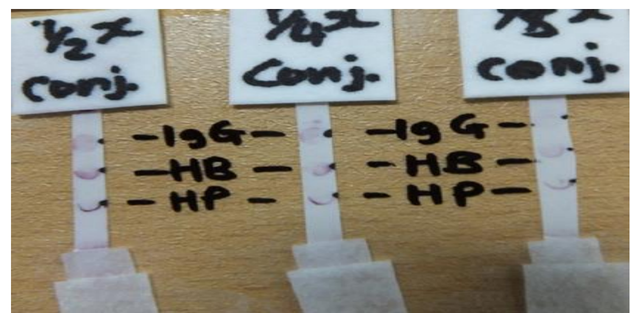



C: 1/10-4x dilution (300ng/ml) D: 1/10-5x dilution (30ng/ml)

**Figure 7: Limit of detection for designs employing chromatography paper conjugate pads. Note: HP: Helicobacter pylori; HB: Hepatitis B; IgG: Human Immunoglobulin G.**




A1 A2




B1 B2 B3

**Figure 8: Conjugate optimization. Pooling of conjugate (A1), conjugate added one by one (A2), optimal conjugate dilution: 1/2x (B1), 1/4x (B2), and 1/8x (B3).**

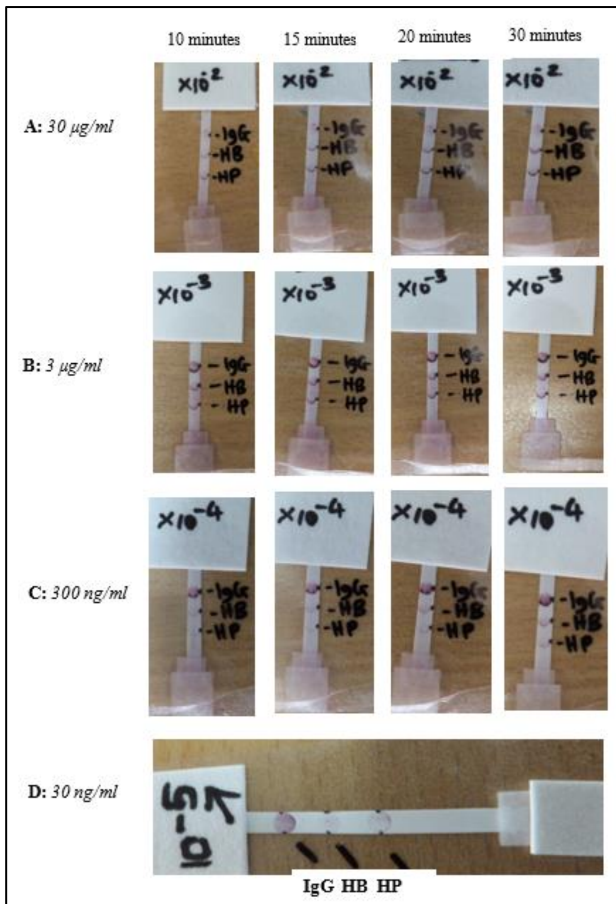



**Figure 9: Limit of detection after 10, 15, 20, and 30 minutes. Note significant signal visible up to 30ng/ml (IgG: Immunoglobulin G; HB: Hepatitis B HP: *H. pylori*).**

One example where multiplexed devices could be of great importance is in the diagnosis of non-malarial febrile illnesses, mostly observed in malaria-endemic tropical countries. In this case, specific identification of febrile etiologies has proven to be a great challenge, forcing clinicians to resort to clinical diagnosis or symptomatic treatment or encouraging a trial-and-error sequential diagnosis of all possible causes of fever once malaria, the most common cause has been ruled out.[18]

Simple diagnostic devices that can help differentiate between malarial and non-malarial fevers simultaneously will greatly facilitate timely management of patients with fever not due to malaria. Other areas of application include detection of antimicrobial residues in animal-derived foods and detection of environmental contaminants in soil and water.

Two multi-arm designs were tested in this study, one that uses glass fiber conjugate pads and the one that uses wax-printed chromatography paper as conjugate pad. Although both designs could show satisfactory test signal results, fiberglass was superior over chromatography paper, as the former afforded complete conjugate release as compared to the later when compared back-to-back. This makes fiberglass a better conjugate pad although chromatography paper offers more versatility for multiplexing due to its wax-patterning potential.

Apart from the above, a single strip multiplex design was also tested using similar analytes and proved to be simple, sensitive and even more convenient than the other two. In this case, there was no difference in signal response whether the conjugate antibodies were pooled before being applied to the conjugate pad or added to the conjugate pad individually (Figure 8A). One common feature for all designs is that despite being able to detect multiple analytes, and with potential for further multiplexing, testing is done through one step only, i.e. the operator needs to add the sample and some buffer, without any more technical requirement. Therefore, such tests can ideally be performed by persons with no advanced training and is thus useful for developing countries where resources are limited.

The limit of detection for tested antigens was found to be 300ng/ml for multi-arm designs, and 30ng/ml for single-strip device. However, lower detection limits can still be achieved if the methodology is slightly modified. Other paper devices tested using different analytes indicated more sensitivity as shown by comparatively lower limits of detection.[30,31]

The most probable reason for reduced sensitivity in this study could be poor concentration of capture antibodies at the nitrocellulose membrane reaction zone due to dispersion of antibody solution around the point of application using handheld pipette. This problem could be overcome if a special lateral flow reagent dispenser or material printer is used to draw compact lines on the membrane instead of hand application. This line will have high and uniform concentration of capture antibodies resulting in a high intensity test signal.

## CONCLUSION

This study demonstrated that a variety of multiplex designs for paper-based rapid diagnostics can be developed to improve diagnosis of common diseases and conditions in resource-limited settings. This approach promises a potential alternative to the more time-consuming and rather expensive sequential testing in circumstances where diseases presenting with similar symptoms cannot be told apart clinically.

Challenges encountered during this designing phase, like low signal will be addressed in subsequent phases by using antibody dispenser for capture antibody application on the membrane. More optimization will be conducted to establish the maximum number of multiple analytes that could be incorporated on one device while retaining the minimum sensitivity and specificity required for point-of-need diagnostic purposes.

## ACKNOWLEDGEMENTS

Authors would like to thank Professor Scott Phillips at Penn State University in whose lab part of this work was done. Gratitude is also extended to the entire Phillips Group, at Penn State University Department of Chemistry for their assistance during this work. We also thank the technical staff at NM-AIST for their cooperation and support during execution of this work.

*Funding: 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*
*Conflict of interest: None declared*
*Ethical approval: Not required*

## REFERENCES

1. Petti CA, Polage CR, Quinn TC, Ronald AR, Sande MA. Laboratory medicine in Africa: a barrier to effective health care. Clinical Infectious Diseases. 2006;42(3):377-82.
2. Yager P, Edwards T, Fu E, Helton K, Nelson K, Tam MR, Weigl BH. Microfluidic diagnostic technologies for global public health. Nature. 2006;442(7101):412.
3. Mabey D, Peeling RW, Ustianowski A, Perkins MD. Diagnostics for the developing world. Nature reviews. Microbiology. 2004;2(3):231.
4. Peeling RW, Holmes KK, Mabey D, Ronald A. Rapid tests for sexually transmitted infections (STIs): the way forward. Sexually transmitted infections. 2006;82(suppl 5):v1-6.
5. Urdea M, Penny LA, Olmsted SS, Giovanni MY, Kaspar P, Shepherd A, Wilson P, Dahl CA, Buchsbaum S, Moeller G, Burgess DH. Requirements for high impact diagnostics in the developing world. Nature. 2006;444(Suppl 1):73-9.
6. Li X, Ballerini DR, Shen W. A perspective on paper-based microfluidics: current status and future trends. Biomicrofluidics. 2012 Mar;6(1):011301.
7. Lu Y, Shi W, Jiang L, Qin J, Lin B. Rapid prototyping of paper-based microfluidics with wax for low-cost, portable bioassay. Electrophoresis. 2009;30(9):1497-500.
8. Yetisen AK, Akram MS, Lowe CR. based microfluidic point-of-care diagnostic devices. Lab on a Chip. 2013;13(12):2210-51.
9. Martinez AW, Phillips ST, Whitesides GM, Carrilho E: Diagnostics for the developing world: microfluidic paper-based analytical devices. Anal Chem. 2010L;82:3-10.
10. Phillips ST, Lewis GG: The expanding role of paper in point-of-care diagnostics. Expert Rev Mol Diag. 2014;14:123-125.
11. Pelton R. Bioactive paper provides a low-cost platform for diagnostics. TrAC Trends in Analytical Chemistry. 2009;28(8):925-42.
12. Mansfield MA. Nitrocellulose membranes for lateral flow immunoassays: a technical treatise. InLateral flow immunoassay. Humana Press. 2009:1-19.
13. Bisoffi Z, Buonfrate D. When fever is not malaria. The Lancet Global Health. 2013;1(1):e11-2.
14. Crump JA, Morrissey AB, Nicholson WL, Massung RF, Stoddard RA, Galloway RL, Ooi EE, Maro VP, Saganda W, Kinabo GD, Muiruri C. Etiology of severe non-malaria febrile illness in Northern Tanzania: a prospective cohort study. PLoS neglected tropical diseases. 2013;7(7):e2324.
15. D'Acremont V, Lengeler C, Genton B: Reduction in the proportion of fevers associated with Plasmodium falciparum parasitaemia in Africa: a systematic review. Malar J. 2010;9:240.
16. D'Acremont V, Kilowoko M, Kyungu E, Philipina S, Sangu W, Kahama-Maro J et al.: Beyond malaria--causes of fever in outpatient Tanzanian children. N Eng J Med. 2014;370:809-817.
17. Seth M, Mdetele D, Phillips ST, Buza J: Challenges in Diagnosis of Febrile Illnesses in Tanzania in the Era of Declining Malaria Epidemiology. Am J Res Comm. 2015;3:88-110.
18. Chipwaza B, Mugasa JP, Mayumana I, Amuri M, Makungu C, Gwakisa PS: Community knowledge and attitudes and health workers' practices regarding non-malaria febrile illnesses in eastern Tanzania. PLOS Neg Trop Dis. 2014;8:e2896.
19. Fenton EM, Mascarenas MR, Lopez GP, Sibbett SS: Multiplex lateral-flow test strips fabricated by two-dimensional shaping. ACS App Mater Interfaces. 2009;1:124-129.
20. Carrilho E, Martinez AW, Whitesides GM. Understanding wax printing: a simple micropatterning process for paper-based microfluidics. Analytical chemistry. 2009;81(16):7091-5.
21. Martinez AW, Phillips ST, Wiley BJ, Gupta M, Whitesides GM: FLASH: a rapid method for prototyping paper-based microfluidic devices. Lab Chip. 2008;8:2146-2150.
22. Bruzewicz DA, Reches M, Whitesides GM: Low-cost printing of poly(dimethylsiloxane) barriers to define microchannels in paper. Anal Chem. 2008; 80:3387-92.
23. Abe K, Suzuki K, Citterio D: Inkjet-printed microfluidic multianalyte chemical sensing paper. Anal Chem. 2008;80:6928-34.
24. Li X, Tian J, Nguyen T, Shen W: Paper-based microfluidic devices by plasma treatment. Anal Chem. 2008;80:9131-34.
25. Ferguson JP, Baxter GA, McEvoy JD, Stead S, Rawlings E, Sharman M: Detection of streptomycin and dihydrostreptomycin residues in milk, honey and meat samples using an optical biosensor. Analyst. 2002;127:951-56.
26. Pikkemaat MG, Rapallini MLBA, Oostra-Van Dijk S, Elferink JWA: Comparison of three microbial screening methods for antibiotics using routine monitoring samples. Anal Chim. 2009;637:298-304.

27. Lewis GG, Robbins JS, Phillips ST. A prototype point-of-use assay for measuring heavy metal contamination in water using time as a quantitative readout. Chemical Communications. 2014;50(40):5352-4.
28. Wang H, Li YJ, Wei JF, Xu JR, Wang YH, Zheng GX: Paper-based three-dimensional microfluidic device for monitoring of heavy metals with a camera cell phone. Anal Bio Chem. 2014;406:2799-2807.
29. Chin CD, Linder V, Sia SK: Lab-on-a-chip devices for global health: past studies and future opportunities. Lab Chip. 2007;7:41-57.
30. Lewis GG, Robbins JS, Phillips ST: Point-of-care assay platform for quantifying active enzymes to femtomolar levels using measurements of time as the readout. Anal Chem. 2013;85:10432-39.
31. Schonhorn JE, Fernandes SC, Rajaratnam A, Deraney RN, Rolland JP, Mace CR: A device architecture for three-dimensional, patterned paper immunoassays. Lab Chip. 2014;14:4653-4658.


**Cite this article as:** Seth M, Buza J. Multiplex paper-based designs for point-of-care (POC) diagnostics. Int J Res Med Sci 2017;5:5052-9.