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Point-of-need diagnostics in a post-Covid world: an opportunity for paper-based microfluidics to serve during syndemics†

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Zoonotic outbreaks present with unpredictable threats to human health, food production, biodiversity, national security and disrupt the global economy. The COVID-19 pandemic—caused by zoonotic coronavirus, SARS-CoV2— is the most recent upsurge of an increasing trend in outbreaks for the past 100 years. This year, emergence of avian influenza (H5N1) is a stark reminder of the need for national and international pandemic preparedness. Tools for threat reduction include consistent practices in reporting pandemics, and widespread availability of accurate detection technologies. Wars and extreme climate events redouble the need for fast, adaptable and affordable diagnostics at the point of need. During the recent pandemic, rapid home tests for SARS-CoV-2 proved to be a viable functional model that leverages simplicity. In this perspective, we introduce the concept of syndemnicity in the context of infectious diseases and point-of-need healthcare diagnostics. We also provide a brief state-of-the-art for paper-based microfluidics. We illustrate our arguments with a case study for detecting brucellosis in cows. Finally, we conclude with lessons learned, challenges and opportunities for paper-based microfluidics to serve point-of-need healthcare diagnostics during syndemics.

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Decentralized healthcare testing can be performed at any setting outside a centralized, high-complexity laboratory: at the patient site (point of care), in the field (point of use) or at home.

Pain-points across all use cases—amply summarized by the REASSURED[1] acronym that was coined by the World Health Organization (WHO)—are the following: (1) real-time connectivity, (2) ease of specimen collection, (3) affordable, (4) sensitive, (5) specific, (6) user-friendly, (7) rapid and robust, (8) equipment-free or simple, and (9) deliverable to end-users. Despite massive strides made during the COVID-19 pandemic,[2] healthcare diagnostics at the point of need (PoN) are still not widely available. The very definition of PoN has different meanings depending on the target audience: academia, defence, government, global non-government organizations (NGOs), or industry. Academics agree that the term is not yet clearly defined.[3] We include here some of the many and varied definitions. The United States' Department of Defense (US DoD) defines PoN as: "*a physical location within a desired operational area designated by the geographic combatant commander or subordinate commander as a receiving point for forces or material, for subsequent use or consumption*".[4] Advanced Research Projects Agency for Health (ARPA-H), a new and disruptive US government agency, describes PoN technologies as: "*those that enable the deployment of critical healthcare resources rapidly, equitably, and securely at scale in permissive and non-permissive (i.e., damaged infrastructure, cyber-denied) environments during a public health crisis or natural disaster*".[5] MRI Global, a world-recognized NGO, describes PoN as testing that can be done without the supervision of the care professional, usually at home.[6] Finally, Yole Group, a leader in insights on microfluidics, beautifully summarized the revolution of PoN testing in their 2023 market report in terms of the industrial ecosystem of available products in the market.[7] They articulated PoN with a succinct definition that we used at the

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very beginning of this paragraph. Finally, our own definition is simple. We use bottom-up thinking, and the outline methodology developed in the Whitesides Group:[8]

- ***What is the problem?*** WHO succinctly defined the problem and its pain-points with the REASSURED acronym. Others have added more nuance to the definition.
- ***What is the solution?*** Tests and devices that provide fast, adaptable and affordable diagnostics in any setting and under any conditions.
- ***Who cares?*** The COVID-19 pandemic led to 30 M lost lives[9] and 7 M directly confirmed deaths.[10] The USC Schaeffer Center for Health Policy & Economics estimated the total macroeconomic cost at $14 T by the end of 2023.[11] Thus, the human and economic toll of lack of PoN diagnostics during outbreaks is so significant that we are care in some way.

Our viewpoint is that paper-based microfluidics are ideal candidates to address the pain-points of PoN healthcare testing, regardless of its definition. We hope that the wider community agrees on the glaring need for pandemic preparedness. In this paper, we go beyond pandemics: We introduce the concept of "*syndemnicity*" and propose ways in which the lab-on-chip (LoC) field can help solve the problem and its pain-points for patients, society, and public health with paper-based tests.

What is syndemnicity, and how can paper-based microfluidics fulfil the need for diagnostics at the point of need?

Pandemics, epidemics & their syndemic effects

Epidemics and pandemics are both examples for outbreaks of infectious diseases.[12] Zoonotic pathogens—like SARS-CoV2 that caused the COVID-19 pandemic— have been the causal agents of most outbreaks in the past century. The recent emergence of avian influenza (H5N1) is a stark reminder of the continued need for outbreak preparedness at the PoN.[13,14] Monkeypox—spreading right now across Africa—[15]is another example of an emerging, zoonotic infectious disease.[16] All these pathogens share common chacteristics,[17,18] which can be leveraged for the development of adaptable diagnostic tests. They are vector-born and are more typically caused by viruses, rather than bacteria. Finally, they are less likely to be transmitted: (1) directly from animals, or (2) environmentally by water or food contamination.

We strongly believe that outbreaks of infectious diseases can no longer be discussed without considering syndemic effects. The term "*syndemics*" was first coined by Merrill Singer, a medical anthropologist, to describe the synergistic combination of substance abuse, violence, and AIDS (summarized in the acronym SAVA) in the 1990s.[19,20] In 2017, the journal Lancet dedicated a full issue to present the syndemics model of health.[21] Post-Covid, Shelke *et al.* described syndemics as new phenomena in human diseases where one or more infectious diseases co-occur with societal, economic and extreme environmental events.[22] They redefined COVID-19 as a syndemic, and not just a pandemic. Similarly, Stucki and co-authors used the same arguments for recommendations to WHO on a disease is classified.[23] This year, Gibb *et al.* discussed how the spread of infectious diseases is fundamentally changing within the context of multi-causal anthropogenic reasons: climate change and socio-environmental drivers.[24] To conclude, there is very little literature on syndemicity, in general. Searching Google Scholar, the term was only mentioned once in the literature in a paper focused on tuberculosis.[25] A more general Google search yields a mere five entries, with only significant addition being a paper of the syndemnicity of COVID-19 by Nigerian researchers Ochu and Wuraola.[26]

Syndemnicity & diagnostics at the point of need

We expand here the terms "*syndemic effects*" and "*syndemics*" to the wider concept of syndemnicity, specifically in the context of infectious diseases and PoN healthcare diagnostics. Not only in resource-limited settings, but also when the environment becomes non-permissive (*i.e.*, damaged infrastructure, cyber-denied) by factors other than the perceived wealth of the country or continent where the outbreak is occurring. Imagine that an earthquake hits San Francisco during a H5N1 epidemic under social unrest caused by political instability, war or due to an economic event, like the 2008 Recession. Healthcare resources will become scarce. Tests and services otherwise performed in centralized labs will no longer be available. Sadly, during the review process of this manuscript in October 2024, another example of syndemnicity occurred. Two hurricanes (Helene and Milton) devastated the Southeast US in two consecutive weeks.[27] The damage included loss of lives and services (power, internet and water). Hospitals had to ration fluids administered to only the sickest of patients leading to the FDA authorizing shipments from overseas plants.[28] Thankfully, a local IV fluid plant, which manufactures 60% of the US supply for IV fluids, was subsequently reported to have been spared after being submerged in water.[27] HHS reported on the 18th of October 2024 that hospitals had 50% more IV fluids available to them than directly after Helene.[29] Similarly, centralized healthcare testing was seriously impacted for weeks after the storms landed.[30] In studies of post-hurricane effects of previous storms, dialysis patients were acutely impacted in the first week after hurricane Sandy.[31] Disruption in diabetes care after Hurricane Katrina motivated clinicians from New Orleans to publish their detailed recommendations stemming from their experiences.[32] In a vicious cycle of syndemnicity, more infectious diseases opportunistically sweep in after storms.[33] Zika, cholera, malaria and chikungunya can be transmitted to humans by infected mosquitoes breeding in floodwater.[34,35] Airborne molds can persist for months in

flooded homes, as observed after Katrina and Rita.[36] Finally, the health impact of extreme environmental events was shown to affect routine healthcare, like prescriptions,[37] in the direct aftermath but also linger for years especially for low-income patients.[38] To conclude, PoN diagnostics can serve communities during and after syndemics, especially while supply chains and infrastructure are still in recovery mode in remote areas with large health disparities due to socioeconomic factors.

Post-Covid PoN diagnostics landscape

We want to start this section by applauding the hugely successful rapid acceleration of diagnostics (RADx) Tech Program for Covid, which ran from 2020 to 2023.[39] The United States' National Institutes of Health (NIH)—with support from Congress—initiated this program in the very early days of the COVID-19 pandemic. Funding was awarded in an expedited fashion with due diligence conducted as in financing rounds from venture capital. Both small and large companies were supported by industry veterans and introduced to manufacturing partners for swift productization. The results were astounding: >7.8B tests and products; 55 FDA-authorized tests; 18 over-the-counter (OTC) emergency use authorizations (EUAs) by the US Federal Drug Agency (FDA); as well as two multiplex tests for COVID-19 and influenza. A detailed document summarizing their recommendations for best practices was recently published by the RADx team.[40]

Different types of bioanalytical assay chemistries can be deployed for PoN diagnostics depending on the analyte of interest. Thanks to Covid, we are all now accustomed to two gold-standard tests, PCR and immunoassays. Polymerase chain reaction (PCR) is a molecular assay chemistry that detects genetic material, either of an infectious pathogen, like SARS-CoV2, or of the patient for genetic screening. Molecular tests are the most accurate and use nucleic acid amplification technologies (NAATs). Immunoassays detect proteins, either viral proteins of infectious pathogens (antigen tests), or the host's own antibodies as part of their immune response to chronic diseases or infections (serology tests). Serology tests have traditionally been better candidates for fieldable applications of diagnostics. However, the COVID-19 pandemic shifted the balance towards point-of-need commercial tests using molecular methods for those who can afford them. More than 246 Covid molecular tests have received regulatory authorization in the US compared to 73 tests using serology and 53 tests directly detecting viral antigens.[41]

The LoC community was instrumental in developing and commercializing diagnostics tests during and after the COVID-19 pandemic. We[42,43] and others[44–46] often have discussed how LoC technologies and microfluidics can fulfil their promise. We should be collectively proud because the promise is now materializing. There are currently two at-home molecular tests marketed for respiratory viruses as consumer products. Lucira Health sells the all-in-one test LUCIRA® by Pfizer COVID-19 & Flu A/B Home Test ($38.93 per test). Aptitude Metrix COVID test retails for a similar price ($24.99 per test) with a separate reader ($49.99). Cue Health successfully productized another at-home, molecular diagnostic test, which reached millions of users. That test is no longer available for reasons discussed later. All three are based on LoC technologies.

Paper-based microfluidics

Now, time to address the elephant in the room: PoN microfluidic tests currently available in the market are simply too expensive as consumer products, in the absence of governmental mandates for testing.[47] And that is where the opportunity for paper-based microfluidics arises. The earliest example of such a test is the modern pregnancy test, invented by Margaret Crane in 1971.[48] It is an at-home immunoassay which occurs on a paper-like strip of nitrocellulose[49] without any power needs, instrumentation or external pumps. Capillarity moves the sample (urine) by lateral flow across different zones. A capture antibody immobilized on the strips captures the pregnancy hormone hCG (human chorionic gonadotropin), if present in the urine sample.[50] More advanced paper-based tests are termed as microfluidic paper-based devices (μPADs). They were established at the US-based academic research group of one the authors of this perspective, George Whitesides, in the mid-2000s.[51–53] The key advantage of μPADs is that they have vertical flow functionality. This allows separation in time and space: (1) assays can be more complex than pregnancy tests; (2) multiple targets can be detected in multi-step reactions. Since then, the field of paper-based microfluidics has exploded with a myriad of academic and industry groups advancing and productizing these first inventions. Many thorough reviews are available.[54–57]

The authors continue to be fascinated by paper and passive materials as substrates for microfluidic devices and LoC technologies. We will first indulge in telling the story of some fundamentals. In 1833, Anselme Payen and Jean-François Persoz first discovered diastase and cellulose.[58] Some 80 years later, first Lucas,[59] then Washburn[60] described imbibition in capillary rise in tubes with the Lucas–Washburn equation.[61] Since then, we are still understanding paper as a substrate, especially its fluid dynamics properties. Brendan McDonald's review on the flow of liquids through paper remains a critical read on flow in paper-based tests.[62] Chang *et al.* applied the Washburn equation in different types of paper.[63] Fu *et al.* explored how transport works in 2-D networks in paper from the test developer's viewpoint, providing with a quantitative framework.[64] More recent work by the research groups of: (1) Sabeth Verpoorte on passive mixing,[65] (2) Jacqueline Linnes on flow dynamics,[66] and (3) Brushan Toley on partial saturation during wicking flow on paper,[67] add significantly to the knowledge base of our field.

Some select developments in the state-of-the-art for paper-based for zoonotic and infectious diseases that excite us

follow. For molecular diagnostics, the Cooper Group in Glasgow (Scotland, United Kingdom) implemented paper microfluidics for highly sensitive detection of hepatitis C virus using loop-mediated isothermal amplification (LAMP).[68] They later demonstrated a more sophisticated paper-based version of their system for molecular detection of COVID-19 in wastewater.[69] Also this year, Jiang *et al.* presented their UbiNAAT platform here on Lab Chip.[70] This system detects SARS-CoV2 and influenza A from a single swab with a sensitivity of 10^4 viral copies per swab. Smith *et al.* developed a multiplex low-cost assay for cervical cancer.[71] They evaluated their test with self-collected samples from El Salvador and Mozambique, as well as performed usability studies. The same lab (Rebecca Richards-Kortum, Rice University) then integrated the assay into a low-cost prototype with a clinically relevant limit of detection of 1000 HPV16 or HPV18 DNA copies per test.[72]

For sample preparation, Z. Hugh Fan's Group showed a paper-based, RNA enrichment device for Zika virus detection within 25 minutes.[73] The Guijt lab at Deakin University (Australia) presented the use of mixed cellulose ester (MCE) paper for DNA binding by ionic interaction under molecular crowding conditions and fluid transport by wicking.[74]

For applications in agriculture, the Verma lab at Purdue University (USA) demonstrated a LAMP-based fieldable assay for detection of bovine respiratory disease (BRD) bacterial pathogens.[75] Chuck Henry and his lab recently presented a μPAD for non-destructive assessment of durian maturing and sweetness.[76] Patel *et al.* developed a prototype for multiplex testing of soil macronutrients in farming relevant ranges.[77]

For purity validation of pharmaceuticals, Oday *et al.* developed and validated a paper-based device for quantifying amoxicillin.[78] More widely, Marya Liberman's lab at the University of Notre Dame specializes in paper-based devices detecting chemicals in environmental samples, illicit drugs and active ingredients purity in pharmaceuticals. She recently published a book chapter on how paper-based tests can help screening of bad quality pharmaceuticals in field settings.[79]

In an interesting twist to more traditional lateral and vertical flow microfluidics, the labs of Dino DiCarlo and Aydogan Ozan combined deep learning and vertical flow assays to detect cardiac troponin I (cTnI) in serum within 15 min per test (detection limit of 0.2 pg mL^{-1}).[80] Other novel approaches include paper-based Raman by Andrea Locke at Vanderbilt University,[81,82] and wearable paper-based devices for sweat analysis developed at the Limei Tian Group.[83,84]

Finally, some elegant and surprising "*coffee-themed*" uses of paper have been demonstrated for cytometry and soil testing, respectively. Murray *et al.* used the humble coffee filter to perform paper-based cytometry of white blood cells with unimpeded transport in both lateral and vertical directions.[85] Nicole Pamme's lab in Stockholm University presented a cafetière-style soil testing system for use in resource-limited settings, such as Kenya.[86] A μPAD was used for visual readout of results, then recorded using a smartphone app.

Infectious diseases

Infectious diseases continue to devastate public health, the economy and society as whole on a global scale.[87] Extreme climate events, like those mentioned before, redouble the need for PoN diagnostics of infectious diseases.[88–90] The field is wide and complex with a myriad of specialist journals, including Infectious Diseases by The Lancet. For the purposes of this perspective, we will briefly review the impact of two blood-borne pathogens causing sexually transmitted diseases (STDs): acquired immunodeficiency syndrome (AIDS) and hepatitis.

AIDS is now a preventable and fully-manageable chronic, infectious STD with antiretroviral therapies (ARTs) for adults.[91] A cure for children was first reported with the case of the Mississippi baby in 2013,[92] and with the NIH recently publishing results of children remaining free of detectable HIV a year after their ART.[93] In late July 2024, the next Berlin patient, or the seventh patient seemingly cured from HIV was reported after a stem cell transplant where the donor was heterozygous for the CCR5/delta32 mutation.[94] But we would be remiss to not highlight the loss of lives[95] and societal anguish[96] during the AIDS epidemic of the 1980s. Sadly and at odds with the goal of "Ending the HIV Epidemic" (EHE) initiative by the US Health and Human Services (HHS) to eradicate HIV in the US by 2030, nearly 40 000 people are newly diagnosed with HIV each year.[97] Of all new infections, the CDC estimates that roughly 80% were caused by people who did not know they were infected.[98] HIV is often syndemic with hepatitis C (HCV) and tuberculosis, as well as other STDs, like syphilis and gonorheea.[99] Similarly to HIV, curative agents in the form of direct-acting antiviral medicines (DAAS) are now available against HCV so that the disease can be eradicated completely in 95% of patients;

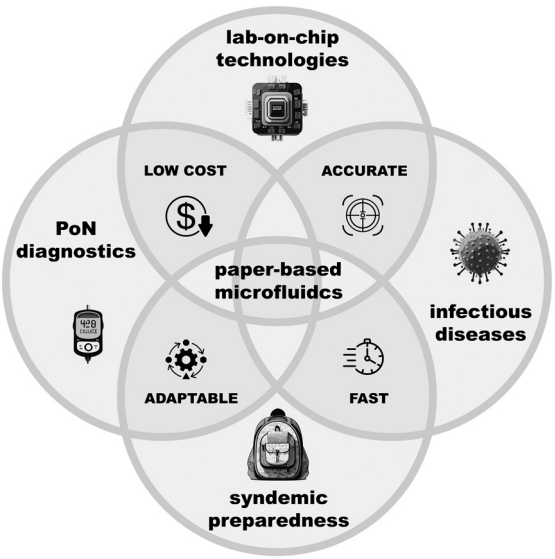

Fig. 1 An overview how paper-based microfluidics are ideally positioned to serve the pain points for PoN diagnostics of infectious diseases during syndemics.

however, a patient must be diagnosed early in order to receive treatment before liver damage becomes permanent.

Paper-based microfluidics are ideal candidates for accurate, low-cost, adaptable PoN healthcare testing of infectious diseases for chronic conditions, as well as for the prevention of disease spread during outbreaks. After the COVID-19 pandemic, needs for low-cost, adaptable, fast and accurate healthcare testing remain unmet (Fig. 1). If such tests become widely available, we may see the day of complete eradication of AIDS and HCV, two major chronic diseases in the US, within our lifetimes. In the next section, we use detection of brucellosis as a case study for how paper-based microfluidics can serve PoN diagnostics for infectious diseases during syndemics.

What did we learn from a cross-continental collaboration in 2014–17 for Brucella detection?

Brucellosis

Brucellosis is a zoonotic, infectious disease, named after David Bruce, who first identified *Brucella melitensis*, a small, Gram-negative bacterium targeting vital organs of mammalian organisms in 1887.[100] It is caused by Gram-negative, coccobacilli bacteria of the genus *Brucella*, and found mostly in domestic and wild animals.[101] These bacteria are highly infectious, especially the *Brucella melitensis*, *Brucella suis*, and *Brucella abortus*, which promote the most severe symptoms in humans.[102,103]

Brucella is classified as a category-B, biological-threat agent by the Centers for Disease Control and Prevention (CDC).[104] Humans can be infected by: (i) direct contact with the tissue of infected animals; (ii) ingestion of contaminated food or water; and (iii) inhalation of infectious aerosols. Brucellosis can cause acute febrile illness such as undulant fever, which may progress to a more chronic form and produce serious complications including musculoskeletal, cardiovascular, and central nervous systems dysfunctions. Although human brucellosis has a very low mortality rate at 5% of diagnosed cases,[102] it is still a neglected disease in the developing world.[105]

Direct or indirect bioassays can be used for laboratory-based detection of brucellosis.[106,107] Direct methods isolate and identify the causative strain of *Brucella*, and include microbiological culturing, and immunohistochemistry. Indirect methods include immunoassays and molecular tests. However, the US Department of Agriculture (USDA) has yet to adopt molecular assays as one of its uniform methods for the eradication of brucellosis in animals such as cattle, bison, and swine.[108,109] The most prevalent PoN test for human and animal brucellosis is the Rose Bengal test (RBT),[110,111] a rapid agglutination assay for antibodies against a ubiquitous, outer membrane sugar of *Brucella* spp. It costs $0.69–0.79,[112] but can yield a high number of false negatives (due to its low sensitivity).[113]

Finally, some vaccines for *Brucella abortus* are available for cattle,[114] as well as a variety of tests for on-farm and clinical diagnosis of the disease to prevent spread. Still, brucellosis is yet to be eradicated. We note here that the COVID-19 pandemic had many common characteristics to brucellosis, with several vaccines and a large menu of diagnostics available.[115]

In the past, *B. melitensis*, *B. suis*, and *B. abortus* were explored as biological weapons.[116] The relative stability of *Brucellae*–in aerosol form, combined with a low infectious threshold–make these agents highly suitable for acts of bioterrorism. *Brucella* could be used to attack both human and animal populations. The most likely form of intentional release would be *via* infectious aerosol; however, food-borne exposure is also possible. Effects of brucellosis in cattle include reproductive complications (late abortion of previously healthy pregnancies and infertility) and reduction of milk production.[117]

To quickly diagnose brucellosis and prevent milk and cattle scarcity due a bioterrorist attack—directly affecting India economically as the world's largest milk producer (31%),[118] and indirectly US citizens at the end of the supply chain—the Defense Threat Reduction Agency (DTRA) of the US DoD, in collaboration with the Indian Army, contracted work for the development of paper-based diagnostic tests for detecting brucellosis at the PoN.

A personal account from this work

Research and development were carried out by the Whitesides Group. We collaborated closely with the Defense Research and Development Establishment (DRDE), an Indian laboratory of the Defense Research and Development Organization (DRDO). DRDE has a mission to design and develop state-of-the-art detection and protection technologies against hazardous materials and microorganisms for national security.

During this project, we developed the following paper-based tests: i) a vertical-flow, paper-based, analytical device for an indirect immunoassay with visual detection by agglutination of gold nanoparticles; and ii) a paper-based, analytical device for an indirect immunoassay with visual detection by polymerization amplification.[119] Detection strategies, device designs and non-embargoed, proof-of-concept results can be found in the ESI† section.

Manufacturing of paper-based diagnostics involves many elaborate steps. The Mace Group (Tufts University) has carefully presented the manufacturing process for prototypes in two separate publications.[120,121] One of those steps is patterning layers of paper and double-sided adhesive in order to create vertical fluidic paths. A laser cutter can be used for this process. This instrument was available to our US-based research lab, but not at DRDE. As a mitigating strategy during the first stages of the project, we prepared individual layers at Harvard and shipped them to India.

In later stages, to allow DRDE to prepare devices independently at their site for this project and beyond, we

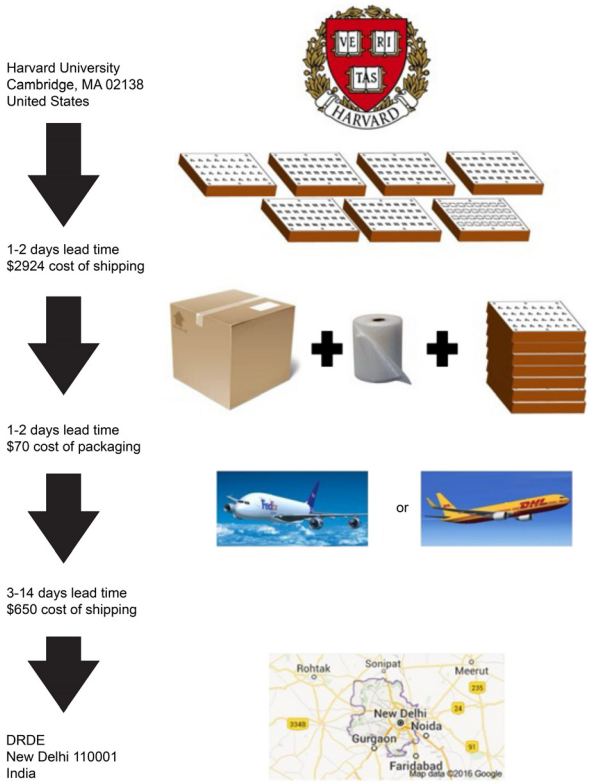

Fig. 2 Logistics of procurement and shipping cutting dies to DRDE in New Delhi (India) from Cambridge, MA in the United States.

designed steel cutting dies for patterning double-sided tape process using a hydraulic press. The advantage of this approach was that we expedited completion of field tests, without compromising our manufacturing processes. The dies were purchased by Harvard in the US, and then shipped to India. This is one of a myriad of instances during that project of how we adapted standard operational processes to achieve our objectives. Fig. 2 summarizes how this seemingly simple step of ordering and shipping dies took almost one month, which was by no means anticipated when preparing our statement of work and timelines when applying for this contract to the DoD.

Overall, the project was large and complex, involving laboratories on two continents, sometimes conflicting bureaucracies, interests that were not always aligned, and different systems of technology development, all with oversight by the US DoD. Execution of such a program often encountered unexpected hurdles, which required continuous improvement of processes. However, we were deeply incentivized with common objectives for preparing against a vitally important threat to both US and Indian citizens and economies. And this led to delivering our key results fully, as well to building strong life-long connections for future research projects. Above all, we gained invaluable lessons on how to make such an international collaboration between dispersed teams a success:

- To seek fundamental understanding of the constraints in each location: instrumentation, personnel expertise and availability of reagents are some of the top considerations at the planning stage of a proposal to any funding organization, governmental or private.
- To develop adaptable, transferable and accessible standard operating procedures. Quality management systems are excellent tools but real-life practicalities of manufacturing for diagnostic devices should also be top of mind. To this end, WHO offers rich resources to help test developers.[122,123]

How can paper-based microfluidics do better and serve society during syndemics?

We present our outlook on specific problems the field should focus on, as well as some challenges and their corresponding potential long-term solutions. As demonstrated by the varied and impressive work highlighted in our earlier personal account of the state of the art, paper-based microfluidics is now mature, some 17 years since the Martinez *et al.* paper was published.[51] And some 53 years since Margaret Crane's patent on the pregnancy test provided future generations of women reproductive freedom in the privacy of their own homes.[48] However, there are still scant examples of paper-based tests available in the market, and no NAAT tests based on paper-based microfluidics.

Academics should continue their efforts to grow the fundamental understanding of paper as a manufacturing material for diagnostic tests. And we should certainly deploy commercialization and productization tools that are used in industry and early-stage startups for:

- **Fabrication.** Patterning with wax printing is no longer a viable option since Xerox discontinued its ColorQube in 2016. Roller *et al.* provide an overview of available options.[124] Laser pyrolysis[125] and laser direct-writing[126] and ink writing using nanocellulose hydrogels[127] are some of the most promising alternatives to wax printing under development. The next step is achieving the Holy Grail of throughput by scalable manufacturing beyond chip-in-an-lab prototypes for academic publications, and batch manufacturing to low production volume numbers. This paper from the Sikes and Mace labs presented a promising RADx-funded pilot manufacturing line using roll-to-roll patterning with UV-curable resins.[119] The process is amenable to manufacturing at a mass scale and includes jetting of reagents—like pregnancy test manufacturing—with layers sealed together using ultrasonic welding.
- **Sample preparation.** Sample preparation and manufacturing of new paper-based test designs (in contrast with old, established lateral flow alternatives) are worthy of attention. Significant more fundamental research is needed, as recently reviewed.[128,129]
- **Target product profile.** Use of the—often underused—target product profile (TTP) as a tool for regulatory communication will greatly facilitate and expedite the path to regulatory clearance for market entry.[130]

• **Cost of goods modelling.** In academia, cost reduction is often a key element of sensor design.[70,131] The pandemic showed us that consumables and reagents for PoN molecular tests are indeed higher than what the market will bear. Use of bill of materials (BOM) and cost of goods (COGS) modelling at the cartridge design stage can reveal hidden and unexpected costs sooner rather than later.

• **Design thinking.** This methodology is used routinely in mainstream consumer product design. Recent work in our field demonstrate how we can use usability studies,[85] and human centered design[132] in paper-based microfluidics.

• **Go to market strategy.** The mantra of venture capitalists and investors of finding product market fit,[133] identifying the best entry marker and business model with customer discovery[134] continues to be the absolute binary criterion for the success of a company, healthcare diagnostics included. Recent bankruptcies of well-funded companies, such as Cue Health and Lucira Health, confirm that making it to market, and past regulatory approval does not guarantee long-term success.

Concluding thoughts

We foresee a shift from serological assays at the point of need towards molecular tests, if costs of molecular tests can be lowered. Going molecular enables rapidly adaptable tests with multiplex detection of pathogens, which is ideal for pandemic preparedness against new strains, and for pivoting commercial products quickly.[135] In addition, molecular assays directly detect viral nucleic acids instead of viral protein and before host response onset, associated with missed positives. For HIV, this is often referred as the "*HIV Window Period*".[136,137] Combined with next-generation microfluidics, the promise of point-of-need diagnostics may soon become a reality. Collaborative efforts between governments, academia and industry can help overcome the most significant hurdles before widespread adoption from consumers, which comes down simply to affordability[47] and ease of use. Working together across different cultures, geographies, and with often conflicting motivations is continually challenging. Despite the human and economic cost of the COVID-19 pandemic, negotiations for Pandemic Preparedness Treaty—spearheaded by the World Health Organization (WHO) for more than two years—failed to be ratified in May 2024.[138]

Paper-based devices can help secure the first beachhead for a point-of-need test that becomes widely adopted by careful design thinking and well-researched go-to-market strategies. Most importantly, it is key to identify underserved populations, during customer discovery. Continued unmet needs persist in women's health, where there is a significantly higher burden for morbidity-driven diseases due to lack of gender-specific diagnostics.[139] Countless health outcomes will be accelerated, including a shortened value chain of patient care and faster decision-making by physicians, leading to lower healthcare costs and enhancing an interconnected healthcare chain. We note that US healthcare expenditure for chronic conditions makes for 17.3% of the federal GDP.[140] Leveraging the low complexity of paper-based microfluidics and high adaptability of molecular diagnostics can lead to a healthier population[141]—with fewer infections and better-managed chronic diseases—as well as serve as rapidly-adaptable test-and-treat tools during emergent outbreaks with the characteristics of syndemnicity.[142,143] Using the "*Heilmeier Catechism*",[144] what if a paper-based test for PoN was widely available for healthcare diagnostics for global outbreaks with the characteristics of syndemnicity? This is our challenge, and we are so close.

Data availability

The imaging data supporting this article have been included either as figures, or as part of the ESI.† No primary research results, software or code have been included were generated or analyzed as part of this perspective.

Author contributions

G. M. W. and H. D. S. secured funding. M.-N. T., D. C. C., J. M., D. T. S., H. D. S. and G. M. W. conceptualized the work and supervised the work. M.-N. T., D. C. C., J. M., K. M., I. C. R., J. I. and S. L. performed experiments. M.-N. T. and G. M. W. wrote the manuscript.

Conflicts of interest

M.-N. T. and G. M. W. are co-founders and have stock in Mitos Diagnostics, Inc. G. M. W. also has financial interests in Mitos Diagnostics, Inc. through the George M. Whitesides 1979 Revocable Trust. Harvard University has financial interests in Mitos Diagnostics, Inc., including licensed intellectual property and stock ownership. H. D. S. has financial interests in Thrixen PTE Ltd.

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