Multiplexed testing for HIV and related bacterial and viral co-infections at the point-of-care: quo vadis?


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Recently, there has been a paradigm shift toward an understanding of the need to screen select sub-populations for several sexually transmitted and blood-borne infections simultaneously, at one time with various rapid point-of-care (POC) technologies, rather than one infection at a time. This is an encouraging and promising change, however many contextual factors need to be considered before implementing such technologies. In this editorial, we highlight some challenges, issues and concerns regarding implementation, integration, and uptake of these technologies across global settings. However, careful planning and well thought out implementation plan that include investments in training health care professionals, improving test and treat algorithms, rapid protocols on communicating actionable results to providers, and timely action, will bring about the desired impact in patient’s lives. This is especially true in settings where they stand to achieve the maximum desired public health and social impact.

KEYWORDS: HIV and co-infections • multiplexed • point-of-care tests • simultaneous testing

Background

The world is facing a significant burden of sexually transmitted and blood-borne infections. The global prevalence of HIV is around 34 million [1], and related key viral and bacterial infections add over 500 million to this estimate [2]. HBV contributes 350 million [3], HCV 170 million [4] and syphilis 36 million [5] to this estimate. Other related infections further exacerbate the existing disease burden. These include HPV, HSV, bacterial vaginosis, Chlamydia trachomatis (CT), Neisseria gonorrhoeae (GC) and Trichomonas vaginalis (TV). In fact, TV, GC and CT alone account for additional 300 million cases [5]. Still, all these global estimates are considered to be conservative approximations. Moreover, many co-infections associated with HIV go unscreened and undetected, and these missed opportunities result in full-blown infections that are costly for patients and health care systems.

Clinical evidence has suggested that the presence of one or more co-infections facilitates HIV transmission and disease progression, and also often leads to mortality [6–9]. These facts point to the need to not only screen high-risk populations for HIV, but also concurrently screen them for all related co-infections. Also, efficient and timely screening (frequent screening after sexual debut and annual screening in areas/sub-populations with high prevalence and incidence) is important to achieve effective treatment of these co-infections.

Screening for co-infections in key at risk populations is thus justifiable. Yet, technological, treatment and resource limitations in global settings sometimes hinder a strategy deployment [10]. In addition, health providers fail to recommend timely screening because they are sometimes unaware of the changing guidelines and of availability of high-quality and – most importantly – affordable rapid point-of-care (POC) tests. So, in both developed and developing settings, patient populations and at-risk clients remain unaware of the need to screen for co-infections.

To address the problem of undetected infections in high-risk populations, many public
health institutes, agencies and government departments have updated their guidelines. Recently, the US Department of Health and Human Services issued guidelines to screen for HIV-related co-infections like HBV and HCV. By highlighting the importance of screening specific sub-populations [11], these guidelines set a precedent for other public agencies to follow suit. The CDC also issued similar guidelines to screen for HCV and HBV in select sub-populations [12-15]. In developed countries, implementing these guidelines would reduce the cost of disease management and improve the health of HIV-infected injection drug users, given that 50–90% of them usually present with HCV co-infection [16]. In fact, with rapid availability of novel and potent regimens for HCV, the vision of control of HIV/HCV is now a reality. As for HBV screening guidelines, these would benefit new immigrants from endemic settings, minority pregnant women and aboriginal populations who face lack of access to quality health and HBV vaccination services. In developing countries, screening for HBV would also offer hope because of widespread availability of Lamivudine. However, many public programs in these countries still do not have resources to afford HCV treatment, and so screening for it remains ineffective in achieving its intended impact. On the other hand, syphilis screening and treatment with the widely available penicillin has proven to be very cost-effective and successful in preventing perinatal deaths. With studies pointing to the need to introduce syphilis tests in global settings to control infection [17], the WHO released guidelines in 2012 to screen pregnant women for both HIV and syphilis ideally in their first trimester to prevent mother-to-child transmission [18-19].

Operationally, in global settings, optimizing the available HIV testing platform to screen for co-infections offers a cost-effective solution with reduced infrastructural and implementation costs. While a combined, simultaneous or multiplexed HIV, HBV, HCV and syphilis POC screening strategy is gaining traction [20], other infections that are on the rise in at-risk populations are also becoming mainstream. For example, POC tests and platform screening tests for related viral infections such as HPV and HSV, as well as related bacterial infections such as TV, CT and GC are beginning to transform the diagnostic landscape, with a hope to catalyze a change.

With accurate multiplexed screening tests and PCR-based confirmatory tests as well as highly effective treatments, infection control would be significantly improved. In this editorial, we discuss the needs, the innovations, the drivers and the future of multiplexed rapid and POC tests.

**Needs for multiplexed POC testing**

Multiplexed tests are ideally suited for marginalized populations that are often lost to follow-up and will thus benefit from early knowledge of the disease state at the point of care. They offer public health advantages as well, by offering an easy screening solution in the absence of clinical suspicion, leading to improved case finding. Furthermore, when the background prevalence of risk factors is high, tests may serve as broad screening tools by detecting infections that may otherwise be missed. So, simultaneous testing of multiple infections using multiplexed POC technologies is a promising infection control strategy that may help delay progression of primary infection, reduce the risk of co-infections and prevent onward transmission of infection. This is especially important when dealing with sexually transmitted infections, given their often asymptomatic or atypical presentation [21]. By improving the turnaround time, such an approach could also potentially increase efficiency by reducing the time to confirmatory testing and treatment initiation with screening. In addition to time saving, a multiplexed test-based strategy could accrue cost savings to the health care systems [17,22,23]. The decrease in cost results from replacing complex and expensive conventional tests, from avoiding complications due to delayed diagnosis of co-infections and from averting antibiotic resistance due to uninfomed treatment assignment [21]. The need for multiplexing is driven by the move toward optimization of already-established HIV platforms of care to offer integrated services for other co-infections and, therefore, improve the quality of health delivery and patient health outcomes. New evidence from the US suggests that, for example, dual HIV/HCV testing programs are feasible to operationalize in clinics that are regularly offering HIV rapid testing to clients [24,25].

Although individual POC tests to diagnose sexually transmitted infections like HCV, HBV and syphilis (some of which have sensitivities and/or specificities greater than 99%) are currently available [26-28], running separate tests for co-infections is both time-consuming and cumbersome. Individual testing requires larger samples of blood, multiple test kits, different controls, buffers and developer solutions, increased labor time and greater number of health professionals.

Combined multiplexed biomarker-based or chip-based POC tests (akin to a POC micro-assay) are innovative [29,30] and may work well in both outreach settings in developed countries and resource-limited settings in developing countries. Several platform-based technologies or portable DNA/RNA-based screening technologies are also in development. They are innovative and offer the promise of confirmatory testing within a reasonable turnaround time. But only time will tell whether or not these will be the affordable POC testing technologies of the future.

**Innovations in multiplexed POC testing**

The global momentum for funding the development of multiplexed POC assays is evident from the funding granted by Bill & Melinda Gates Foundation and Grand Challenges Canada. These agencies recently awarded US$31 million in grants for the development of a simple, multiplexed platform to detect multiple diseases at the point of care [31]. More recently, the X Prize Foundation announced the Qualcomm Tricorder X PRIZE, a US$10 million global competition to stimulate integration of precision multiplexed diagnostic technologies [32]. Indeed, many technologies have been used in the making of multiplexed tests, such as microfluidics, nanotechnologies, impedance technologies and magnetic resonance imaging, rendering it an exciting new area of development.
In this ever-expanding repertoire of assays, multiplexed biomarker-based assays take singleton rapid simplistic POC testing to the next level. These assays promise to detect several biomarkers (i.e., antigens and antibodies) for common HIV-related co-infections within a few minutes, using a single disposable test cartridge and a small fingerstick sample [33]. The most used technologies that have been developed to simplify these multiplexed devices and render them operational at POC are the immuno-chromatographic lateral flow, the flow-through technology (vertical flow) and the Dual Path Platform (DPP) technology. A recent study evaluated three multiplexed rapid diagnostic tests that employ three different technologies: Standard Diagnostics BIOLINE HIV/Syphilis Duo (lateral flow), Medmira multiplex HIV/TP (vertical flow) and ChemBio DPP HIV-syphilis Assay (DPP) [34]. All three rapid diagnostic tests were found to perform well when compared to confirmatory standard tests and to have high specificities and sensitivities [34]. Still, in terms of operationalization, the vertical flow has some comparative advantages over the lateral flow, given that it does not require timed steps (less training needed) and gives the results faster [35]. The new DPP technology also provides results quicker than the lateral flow, but more importantly, it shows improved sensitivity [34,36]. Nonetheless, Standard Diagnostics BIOLINE HIV/Syphilis Duo has been evaluated in six countries and has been proven time and again to have high (99%) sensitivities and specificities [37]. Combining multiple tests continues to be proven achievable with the introduction of rapid multiplexed POC tests that can simultaneously detect three infections. Examples include Medmira’s Multiplex HBC/HIV/HCV [38], which is currently being evaluated in many countries, and Chembio’s DPP HIV/HCV/Syphilis, which was shown to have sensitivities and specificities above 90%, except for the syphilis test’s sensitivity (44%) [39]. Many other multiplexed POC devices that are biomarker based (e.g., smartphone-based or fabric-based) are under development today [40] and are being adapted to many infectious pathogens (other than HIV and co-infections).

Other promising innovative assays that are under development include proteomic, genomic-based and chip-based devices, and some of them have been tested in the field [41]. The chip-based assays have an advantage over the aforementioned visually interpreted assays (lateral and vertical flow assays and DPP technology) in that they rely on an instrument to objectively display results. By eliminating the user’s subjective interpretation of results, these new tests can reduce the risk of false positives (falsely interpreting the intensity of the reactive band) and, therefore, expedite clinical decision-making at the point of care [41]. Likewise, DNA and RNA detection based devices are also in the production pipeline [33]. These devices have the potential to revolutionize POC diagnostics in the near future with confirmatory results. Where will these devices be most beneficial? Which points of clinical pathways will require them the most? When will they need to be used? What incremental benefits do they offer in the marketplace? How will health systems in both developed and developing settings absorb them? These are some lingering questions that are still open to reflection and discussion.

Interestingly enough, in parallel, some companies are also developing multiplexed near-POC or near-patient testing platforms – many of these are based on immunoassays (e.g., Bio-Merieux VIDAS/miniVIDAS [42], microarrays (e.g., Axygen Ziplex System [43]) and PCR (e.g., Cepheid GeneXpert [44]). Some systems like GeneXpert – which includes high-sensitivity and specificity CT/NG testing [45] in its system and is expected to add HIV, HBV and HCV in 2014/2015 [46] – have the capacity to run several screening or confirmatory tests for different pathogens or biomarkers simultaneously. Cartridges for running dual or single pathogen detection are also available. The sensitivities and specificities of these tests have exceeded those of conventional confirmatory assays. These platforms could be placed in central labs or peripheral labs attached to clinics in urban and peri-urban settings. In the near future, these could also be installed in mobile vans that will allow them to reach millions of individuals in outreach settings worldwide.

Many multiplexed rapid diagnostic tests and platforms have been and are being developed. The US FDA has already approved several of the new technologies to screen for co-infections and many are currently undergoing clinical trials to gain FDA approval. However, other health agencies are not being as active as the FDA in granting approvals. As a consequence, many individuals continue to transmit infectious co-pathogens to their partners and children. It is therefore not surprising that over the past 20 plus years, even with routine screening, the majority of individuals infected with HIV worldwide are unaware of their disease state [47]. The lack of knowledge on disease status is probably even worse when considering co-infections that often have an asymptomatic presentation, but due to lack of public health screening, precise data are unavailable. This trend prevails in both developed and developing settings.

Drivers of multiplexed POC testing

The needs to develop these tests will be driven by various factors: burden of infectious diseases, needs of patient populations, use of rapid test results for clinical decision-making, cost and efficiency, and the capacity of health systems to absorb them for screening purposes.

Disease burden in sub-populations

The development of better screening solutions would benefit mostly marginalized and high-risk populations worldwide. These include infection drug users, men who have sex with men, commercial sex workers, sexually transmitted disease clinic attendees, rural pregnant women, infants and incarcerated populations. In developed settings, immigrants and ethnic populations would also benefit greatly. As a matter of fact, screening for sexually transmitted infections remains uncommon in Canada’s aboriginal populations – either not offered or not accessible – while the incidence is on the rise [48]. HIV and related co-infections have disproportionately impacted all of these high-risk populations, many of which are often unaware of their co-infection disease state [49-52]. The decision to screen these sub-populations should take into consideration the
underlying burden of disease and the need to control it. For example, for infection drug users, it makes sense to screen for HBV and syphilis, in addition to HCV and HIV. For immigrant populations, screening for HBV is important because many immigrants come from endemic settings. In pregnant women, screening for many bacterial (TV, CT and GC) and viral co-infections (HPV, HSV and HBV) is pertinent because of the downstream implications of transmission to their infants and partners. Screening for sexually transmitted bacterial infections is neither frequent nor mainstream; therefore, it is welcomed in both developed and developing settings.

While developing screening solutions, it may be reasonable to devise socially sensitive strategies, because in addition to the growing burden of co-infections, these populations often live in poverty, are discriminated against, and face stigmatization and barriers in accessing care. They also often present late for treatment and care, which further increases the risk of morbidity and complications, and thereby increases the costs to health care systems. In tandem, generating awareness at the population level on the need to screen for them will also be important.

**Needs of patients**

The perspective of the patients is important to not only understand patients’ needs, but also to empathize with their concerns and help alleviate them. Offering to screen for all infections at one time saves time, but patients need to be mentally prepared to receive test results for three or four co-infections. Besides, the number of visits needed to receive confirmatory test results and the ensuing number of tests to be performed will also matter to the patient. Although a convenient sample (like oral or urine) is often used for screening, such biomarker-based multiplexed tests require confirmatory tests, for which venous blood samples are required for each separate infection. However, one might wonder if having to provide numerous samples is truly convenient for the patient. Reducing the amount of blood sample needed for confirmatory testing will certainly help in getting a buy-in from the patient. Besides, for patients, screening is not the only concern. Treatment and referral must also take into account patient preference to ensure success with greater buy-in and fewer losses to follow-up.

**Use of rapid test results**

The aim of POC testing initiatives is to expedite treatment delivery for the newly infected, with partner notification and screening/treatment plans for partners, and counseling and repeat testing plans for test negatives. With integrated platform-based testing solutions, the communication of test results to the providers, the storage of test results and the action plans will be streamlined. But managing this diagnostic process requires a committed lab or diagnostic professional. It is, therefore, important to engage providers especially since they are often not thrilled when a new technology disrupts their clinical practice workflows. It is also crucial to have protocols, algorithms and treatment in place, so that the full benefit of detection of new infections is achieved. If these are not in place, then the process will not be well perceived by patients and the whole purpose of multiplexing will be lost.

**Cost & efficiency of devices**

Some of the POC technologies are battling the offer of a sophisticated all-inclusive testing solution versus a first step screening or triage solution. It is difficult to decide which technology will work best in which setting, and so this will be navigated by trial and error. For optimum health impact, these technologies (simple vs sophisticated) need to be integrated within health systems or used in private markets (clinics, labs and facilities), keeping in mind that various market forces will drive their rate of absorption.

In this global downturn in economy, health systems face budget cuts and aim to optimize resources with minimal increment in cost. By delivering accurate results for several infections in less time, multiplexed tests may help expedite treatment initiation for HIV and co-infections, thus providing a cost-saving solution. However, this value proposition offered by a new solution needs to be weighed carefully. If these tests are offered at low cost (e.g., through subsidization) in public systems, they have the potential to disrupt conventional lab test models and avoid delays in turnaround time that predict patients’ loss to follow-up [53,54]. In an analysis for dual HIV/syphilis POC testing of pregnant women, the total disability adjusted life years avoided amounted to 200,000 in Rwanda [41].

In another analysis by Gift et al. for Chlamydia [23], rapid near-patient POC testing was found to be more efficient when compared to lab-based conventional testing, given its ability to treat more patients and have higher return rates. However, if the downstream tests are not taken care of by the health care systems, then unless they are made very affordable, this alternative solution may not be very cost-effective. Similarly, platform-based POC tests are promising, but they need to be made cost efficient – bulk purchase of test cartridges for the purpose of screening will help bring down the prices per test.

**Future of multiplexed POC testing**

**Future research:** implementation & qualitative

Reviews of technologies and of performance reliability, reproducibility and accuracy exist and are deemed important [22,33,41]. Also, recent data suggest that POC tests are comparably accurate to first-line conventional laboratory tests [26]. But while trials for FDA approvals on multiplexed biomarker-based, gene-based and DNA-based devices are currently underway in the US, research data on the feasibility of multiplexed biomarker-based, gene-based and DNA-based devices are currently underway in the US, research data on the feasibility of multiplexed strategies implementation are very limited [20]. Piloting these technologies in simulated environments or in real-world settings can help prepare them for a successful implementation and uptake of these multiplexed devices. Feasibility, clinical effectiveness, applicability, impact and downstream implementation issues will thus need to be studied in different contexts and settings to help envision how these technologies can be integrated in different countries’ health systems. Also, most diagnostic studies rarely address real-world outcomes, such as impact on health.
Key issues

- Multiplexed POC testing offers a promising and efficient screening solution to infection control strategies – especially for marginalized and high-risk populations worldwide – at a presumably affordable infrastructural and implementation cost.
- There are many versions of multiplexed tests on the market – biomarker-, chip-, platform- and portable DNA/RNA-based multiplexed POC or near-POC screening technologies that are available on the market and that have a potential to perform very well compared to confirmatory standard tests.
- If offered at a low price, POC multiplexed tests have the potential to disrupt conventional screening models and prevent patient loss to follow-up by reducing the turnaround time.
- The need to develop POC multiplexed tests is driven by the burden of disease in sub-populations, the needs and preferences of patient population, their usefulness in clinical decision-making, their cost and efficiency, as well as their capacity to be integrated in health systems.
- There is a need for implementation and qualitative research, as well as for proper integration of POC multiplexed tests in health settings with emphasis on staff training, quality control and assurance, effective communication of results, and action plans for clinical management.

Expert commentary & five-year view

Multiplexed POC testing offers a huge potential to improve precision in diagnosis and monitoring of infections that will minimize overtreatment, reduce resistance and improve the overall quality of care. It will also engage populations in their own care and allow for screening for infections that patients do not routinely get screened for in conventional settings. It will be most cost-effective if offered and targeted to specific sub-populations with the highest burden of disease. However, a lot of downstream implementation issues including integration and cost effectiveness, in resource-rich versus resource-constrained settings, remain underexplored for many of these promising technologies.

In view of the future realities associated in integrating these assays within the health systems that they intend to change, factors such as affordability of tests or cartridge, robustness of use, ease of use, actionable results, and expedited communication of results to providers, will predict their future success.
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