

# BMJ Open Will a quadruple multiplexed point-of-care screening strategy for HIV-related co-infections be feasible and impact detection of new co-infections in at-risk populations? Results from cross-sectional studies

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## ABSTRACT

**Objectives:** Multiplexed point-of-care (POC) devices can rapidly screen for HIV-related co-infections (eg, hepatitis C (HCV), hepatitis B (HBV), syphilis) in one patient visit, but global evidence for this approach remains limited. This study aimed to evaluate a multiplex POC testing strategy to expedite screening for HIV-related co-infections in at-risk populations.

**Methods:** A multiplex strategy was developed with two subsequent versions of an investigational device Miriad. It was evaluated in two non-comparable settings and populations in two countries for feasibility of conduct, detection of new infections, preference and accuracy. Version 1 was evaluated in 375 sexually transmitted disease clinic attendees in Mumbai, India; version 2 was evaluated in 119 injection drug users in Montreal, Canada.

**Results:** Feasibility (completion rate) of the multiplex strategy was high (86.1% Mumbai; 92.4% Montreal). A total of 170 new infections were detected in Mumbai (56 HIV, 75 HBV, 37 syphilis, 2 HCV) versus 2 in Montreal. Preference was 60% in Mumbai and 97% in Montreal. Miriad version 1 specificities were high: HIV 99.7% (98.3% to 100%), HBV 99.3% (97.6% to 99.9%), HCV 99.7% (98.5% to 99.9%), syphilis 85.2% (80.9% to 88.8%); sensitivities were as follows: HIV 100% (94.8% to 100%), HBV 13.3% (6.6% to 23.2%), HCV 50% (1.3% to 98.7%), syphilis 86.1% (70.5% to 95.3%). With version 2, specificities improved: HIV 100% (97.2% to 100%), HBV 100% (97.3% to 100%), HCV 85.3% (73.8% to 93.0%), syphilis 98.1% (93.3% to 99.8%); sensitivities were: HIV 100% (47.3% to 100%), HCV 80.4% (66.1% to 90.6%), syphilis 100% (22.4% to 100%).

**Conclusions:** A quad multiplex POC strategy for HIV and co-infections was feasible to operationalise and preferred by patients in both settings. Many new infections were identified in Mumbai and accuracy improved with version 2 of the assay. Such a strategy will help expedite screening for co-infections,

## Strengths and limitations of this study

- A multiplexed point-of-care test-based strategy for HIV-related co-infections was feasible to conduct in two settings with diverse infrastructure and patient populations.
- A multiplexed test that detects co-infections was greatly preferred and accepted by patient participants in both settings.
- Acceptability for the strategy was also high, suggesting the need to introduce these tests in settings where rapid screening for all co-infections is desired by patients in a single visit.
- Multiplexed screening devices of high accuracy are the need of the hour and confirmatory multiplexed tests will be a great addition in this direction.
- Convenience sampling of participants, and use of an improved device for Montreal study, were key limitations.

particularly where baseline screening is low. These findings are valuable to practitioners, researchers, policymakers and funders involved in initiatives for all four diseases with implications for scale-up.

## INTRODUCTION

The combined disease burden for HIV and related co-infections worldwide is estimated to be 594 million: HIV contributes 34 million, and co-infections such as hepatitis B (HBV) contribute an additional 350 million, followed by hepatitis C (HCV) infected individuals at 180 million and lastly, individuals with syphilis infection stand at 30 million.<sup>1–4</sup> With a large share of cases going undetected,

the true burden of the co-infections can at best be wishful guesstimates. Multiple barriers have impeded efficient screening for HIV, even after two decades of rapid test-based HIV testing and counselling, and the situation is also below par for co-infections. Even though HIV screening is offered more routinely than other co-infections, only about 50–60% of individuals living with HIV are aware of their serostatus.<sup>5</sup> Often, screening for HIV and co-infections is impeded by fear of social visibility, stigma and discrimination, and at other times by long waiting times in clinics, loss of one working day and social visibility associated with testing in public settings. A lack of mandated public health programmes in global settings that offer timely screening and surveillance limit an accurate estimation and timely screening. Further, marginalised populations that bear the brunt of the co-infections epidemic also face barriers such as stigma and discrimination that impede timely engagement in care.<sup>6</sup> Furthermore, populations such as injection drug users (IDUs) face additional barriers with respect to access to health services. These hard to reach, marginalised populations including men who have sex with men (MSMs) in part due to their lifestyle and due to cultural oppression remain hidden from accessing health services.<sup>7–9</sup> All of these factors together further impede access and engagement in screening initiatives.

In global settings, although sexually transmitted disease (STD) clinic attendees are screened for HIV routinely, screening for co-infections such as HBV and HCV is costly and usually not borne by the healthcare systems. As for syphilis screening, despite it being offered for free, timely notification of test results and initiation of linkages to confirmatory testing and treatment are often delayed or not performed diligently. This results in losses to follow-up of screened populations. Losses to follow-up are also relevant to the HIV care cascade and analogous dropouts exist in HBV and HCV care cascades as well. Syphilis is on the rise in many at-risk populations globally. However, its treatment is inexpensive and effective. Besides, in the era of rapidly transforming and improving HCV therapies, a method for rapid and early diagnosis of HCV would offer individuals a chance to enter HCV care earlier. A recently launched UNAIDS-led diagnostic access initiative established a 90-90-90 target whereby 90% of people living with HIV get adequately diagnosed, 90% of those diagnosed get sustained access to effective antiretroviral drugs and 90% of the treated patients achieve a long-lasting low viral load by 2020.<sup>10</sup> In the light of this new target, there is even more of an imperative global need for an improved diagnostic strategy that integrates simultaneous and same day point-of-care (POC) screening, notification, linkages to confirmatory testing and treatment referrals, to optimise test efficiency and thereby impact control of HIV and co-infections.<sup>11</sup>

Diagnosis remains a critical step in infectious disease control,<sup>12</sup> highlighting the need for timely targeted co-infections screening in at-risk populations.<sup>13–15</sup> While

syphilis facilitates HIV transmission, HIV/HCV and HIV/HBV co-infections facilitate disease progression to liver failure, cirrhosis or death.<sup>16</sup> A timely diagnosis of HIV and HCV and HBV co-infections can minimise downstream adverse health effects, offset rapid disease progression, encourage cure and, most importantly, reduce transmission to partners and children. These will cumulatively decelerate co-infection epidemics.

India's absolute HIV burden in young adults is estimated at 2.5 million, the third highest in the world.<sup>17</sup> The STD clinic attendee population is comprised of young high-risk migrants, commercial sex workers (CSWs) and labourers who have paid for sex with CSWs.<sup>17</sup> Integrated Counselling and Testing Centers (ICTCs) conduct voluntary HIV testing, but limited screening for co-infections. Canada, a low prevalence setting, has a total burden of 71 000 infections,<sup>18</sup> and the bulk of the epidemic is concentrated in MSMs, IDUs, CSWs, immigrants and young women. About 13% of the IDU population is HIV seropositive, and about 25% remain unaware of their serostatus.<sup>9</sup> About 88% of the HIV-positive IDUs have a history of being infected with HCV.<sup>9</sup> As for syphilis, the number of cases is on the rise since 2000, with 539 new cases reported in 2010.<sup>19</sup> Although co-infection screening is offered in community clinics, same day POC-based combined test and treat programmes are not a reality yet in Canada, and evidence on the feasibility of operationalising such a strategy is limited.<sup>13–20</sup> Although several new multiplexed POC devices are ready to be introduced into the market, yet real-world data on feasibility of operationalisation and impact beyond laboratory accuracy are needed before these strategies could be safely implemented.

In this context, we set out to determine whether a multiplex screening strategy built around an investigational quad multiplexed rapid POC test was feasible, preferred to the conventional strategy, and, most importantly, if it improved case finding/detection of HIV and co-infections with linked confirmatory testing and follow-up (notification), even in the absence of clinical suspicion. In this report, we describe our evaluation of such a strategy in two diverse non-comparable settings and two diverse and distinct subpopulations who may benefit from such a strategy while living and working within two extremes of healthcare systems and infrastructure in India and Canada. We recruited IDUs in Canada and STD clinic attendees in India, because both were at high risk of contracting, harbouring and transmitting co-infections.

## METHODS

### Study design and objectives

Two separate cross-sectional studies were conducted in Mumbai and Montreal over 18 months (from February 2011 to January 2012 in Mumbai, and from October 2011 to August 2012 in Montreal). The studies were approved by ethics review boards based at the McGill

University Health Centre, and at the participating hospitals (ie, P.D. Hinduja National Hospital and Medical Research Centre (Mumbai), Sion Hospital (Mumbai) and Centre de recherche et d'aide pour narcomanes (CRAN; Montreal)).

Version 1 was evaluated in Mumbai and version 2 was evaluated in Montreal. Version 1 and version 2 were evaluated linearly because an improved version of the assay was developed over time, with an improved buffer solution, and better refined capture agents that were eventually evaluated in Montreal.

Our study objectives were to: (1) estimate feasibility, defined as completion proportion of the multiplex strategy further quantified as of all those who consented to test, how many completed the strategy?; (2) estimate impact, defined as detection of new infections over the study period. New infections were defined as previously undiagnosed infections (includes, but are not limited to acute infections) and are based on the patient's self-report of not having prior knowledge of diagnosis of a particular disease; (3) evaluate strategy preference (multiplex vs conventional). *Preference*: defined as the proportion of study participants who preferred the multiplex strategy over the conventional laboratory-based strategy. Preference consists of a numerator that was defined as the number of participants in the study who preferred multiplexed over the denominator was defined as the total number of participants in whom the strategy was evaluated. Preference is a proportion. Its numerator is defined as the number of participants in the study who preferred multiplexed strategy; and its denominator is defined as the total number of participants in whom the strategy was evaluated. Other measures such as seropositivity (number of positives for each infection, confirmed by the reference standard) and preference for turnaround times were also collected and computed (refer Results section).

STARD guidelines were followed in reporting our results.<sup>21</sup>

### Eligibility criteria

Participants were eligible if the following criteria were met: (A) adult of at least 18 years of age; (B) with an at-risk profile but asymptomatic (ie, sexually active, injecting drugs, commercial sex, more than one sexual partner; recipient of blood transfusion); and/or (C) presenting signs or symptoms for any of the four target infections (ie, HIV, HCV, HBV, syphilis).

Participants were excluded if they: (A) were unable to provide informed consent; (B) had an acute condition requiring hospitalisation; (C) were unwilling to be contacted or (D) were pregnant or breast feeding.

### Definition of a multiplex strategy

The multiplex strategy was built around the investigational test device Miriad Rapid TP/HBV/HIV/HCV Antibody Test Miriad (MedMira Inc., Halifax, Canada; see online supplementary figure S1). This rapid vertical

flow POC test can simultaneously screen for HIV and three co-infections (HBV, HCV and syphilis) with one drop of blood. Results are available within 3–5 min with each biomarker result shown in distinct regions of the test window, allowing for differential diagnosis of the four infections.

For the Montreal study, a new version (version 2) of the multiplex device was made available by the manufacturer. The manufacturer indicated that the new version was produced using an improved buffer solution which had been further optimised to improve simultaneous detection of antibodies to all four infectious agents.

In terms of execution, the multiplex strategy consisted of two visits (figure 1) of about 30 min each. In the first visit, a combined pretest counselling session on all four infections and information on the benefits of the multiplex strategy was offered, followed by a blood draw by venipuncture (phlebotomy) for confirmatory testing and testing with Miriad. Phlebotomised venous blood was inputted into the MIRIAD device.

A semistructured questionnaire was administered to collect demographic characteristics and risk factors data.

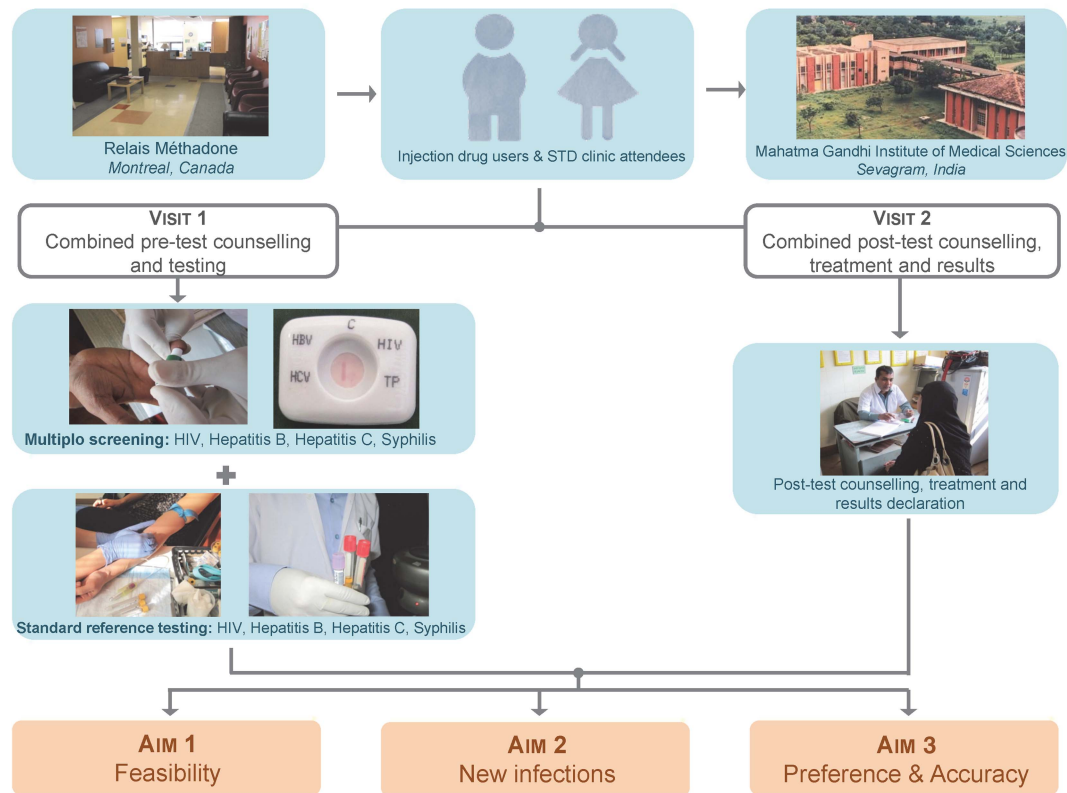
In the second visit, test results were declared, post-test counselling was offered, and treatment and referrals to specialists and centres were arranged. Since the test was an investigational device, results were only made available to the study participant in the second visit, after availability of the confirmatory results from the laboratory. Confirmatory testing was performed according to the guidelines, and paid for by the study when not covered by the health systems (please refer table 1 for testing algorithms for each infection and site).

In Mumbai, multiplex testing was performed and interpreted by a phlebotomist and a physician independently, each being blinded to the rapid test results obtained by the other.

In Montreal, a research nurse performed multiplex testing once. Multiplex POC test results from both sites were classified as preliminary 'positive/reactive', 'negative/non reactive' or 'invalid' for each of the four biomarkers, according to the manufacturer's instructions.

### Data analysis

Data were entered in Excel and exported into SAS software for analysis. The main outcomes evaluated were completion rate, new infections, seropositivity, preference, concordance (in Mumbai) and diagnostic accuracy. Completion rate (feasibility) was defined as the number of participants who completed study procedures that included testing (multiplex and confirmatory), pretest and post-test counselling, and declaration of results over the total number of participants that consented. Impact was computed as the number of new infections identified over the total number of consenting participants. Preference was documented as a proportion with 95% CIs through the questionnaire. Diagnostic accuracy was estimated using sensitivity, specificity and predictive values with 95% CIs calculated from



**Figure 1** Overview of the multiplex strategy in Mumbai and Montreal.

the binomial distribution and assuming laboratory results as the gold standard.

## RESULTS

Results from each site have been described separately below (please refer to the flow of participants in online supplementary figures S2 and S3). Demographic, screening history, risk factors, seropositivity, accuracy and concordance results are reported in [tables 2 and 3](#). It should be noted that as of 2013, the Miriad device evaluated in this study is not in production; other multiplexed

devices such as the triple HIV/HCV/HBV and the duplex HIV/syphilis devices are being manufactured.

### Results from the Mumbai cohort

In Mumbai, 500 consenting participants with suspected HIV, HBV, HCV or syphilis infection were evaluated, of which 125 dropped out after the study procedure was explained to them. As a result, 375 participants were enrolled and completed post-test counselling; of these, 52 participants did not complete their second visit. Confirmatory test result and action plans and referrals were communicated and arranged for 323 participants.

**Table 1** Table of algorithms used for confirming multiplex tests in Mumbai and Montreal

Infection	Mumbai	Montreal
Preliminary positive		
HIV	ELISA (antigen+antibody)+western blot	ELISA+western blot
HBV	HBsAg+total anti HBc+HBV DNA	HBsAg+anti HBc+HBV DNA
HCV	HCV antibody+HCV RNA	HCV antibody+HCV RNA
Syphilis	TRUST+TPHA	VDRL+TPPA
Preliminary negatives		
HIV	ELISA	ELISA
HBV	HBsAg+anti HBc	HBsAg+anti HBc+HBV DNA
HCV	Anti HCV screening test (EIA based)	HCV antibody+HCV RNA
Syphilis	TPHA	VDRL

HBC, HB core; HBsAg, HB surface antigen; HBV, hepatitis B; HCV, hepatitis C; TPHA, *Treponema pallidum* haemagglutination assay; TPPA, *Treponema pallidum* particle agglutination assay; TRUST, toluidine red unheated serum test; VDRL, venereal disease research laboratory test.



**Table 2** Table of demographic and risk factors data from STD clinic attendees in Mumbai and IDUs in Montreal

Category	Mumbai	Montreal
Preliminary positive		
Population	375 STD clinic attendees	118 IDUs
Gender	83.0% males	67.9% males
Age (mean)	31.2 years	38.3 years
	(19–63 years)	(21–62 years)
Previously tested for (baseline screening by conventional testing methods)		
HIV	48.0%	96.3%
HBV	1.6%	83.8%
HCV	1.9%	94.3%
Syphilis	2.7%	58.7%

HBV, hepatitis B; HCV, hepatitis C; IDU, injection drug user; STD, sexually transmitted disease.

In Mumbai, participants presenting to the sexually transmitted clinic were younger (mean age 31.2 years, predominantly male (83%; for details, refer [table 2](#)). As per verbal reports, at baseline, only 48% of individuals had previously been screened for HIV, 2.7% for syphilis and less than 2.0% for HBV and HCV.

In terms of feasibility, the completion rate for the multiplex strategy was 86.1% (323/375), with 52 participants not completing their second visit. About 60.2% (226/375) of participants expressed a preference for multiplexed versus conventional testing. Overall, about 99.5% (373/375) participants were satisfied with their overall testing experience, and 33% (125/375) were willing to recommend multiplex testing to a friend. When asked about the preference for turnaround time for results (TAT), about 43% (161/375) expressed a desire to receive results within a day and 31% (115/375) were willing to wait up to a week.

With Miriad results confirmed according to gold standards (refer [table 1](#)), about 14.9% (56/375; 95% CI

13.1% to 16.7%) of participants were diagnosed with HIV, 20.0% (75/375; 95% CI 18.0% to 22.0%) with HBV, 9.9% (37/375; 95% CI 8.4% to 11.4%) with syphilis, and about 0.5% (2/375; 95% CI 0.2% to 0.9%) with HCV. In all these cases, patients had no prior knowledge of infection.

Regarding diagnostic performance, compared with gold standards, specificity estimates for Miriad (version 1) were: HIV 99.7% (95% CI 98.3% to 99.9%), HBV 99.3% (95% CI 97.6% to 99.9%), HCV 99.7% (95% CI 98.5% to 99.9%) and syphilis 85.2% (95% CI 80.9% to 88.8%). Corresponding sensitivity estimates were: HIV 100% (95% CI 94.8% to 100%), syphilis 86.1% (95% CI 70.5% to 95.3%), HCV 50.0% (95% CI 1.3% to 98.7%) and HBV 13.3% (95% CI 6.6% to 23.2%). High negative predictive values were found for all four infections, while positive predictive values varied with wide CIs for co-infections. No co-infections were identified in the study sample.

### Results for the Montreal cohort

In Montreal, 155 participants were approached for participation, of whom 37 were not eligible as they did not have a Medicare card. Of the remaining 118 participants who were enrolled, 9 did not complete the study procedure because of difficulty with obtaining blood with phlebotomy—as a consequence, 109 participants completed the study procedure. In Montreal, participants were IDUs, predominantly males (68%) and middle-aged (mean age: 38 years; details refer: [table 2](#)), with a very active history of screening for HIV (96%), HCV (94%) and HBV (84%) compared with syphilis (59%) compared with the Mumbai cohort. Feasibility of the strategy defined by the completion rate was 92.4% (109/118). Compared with the gold standard, seropositivity of infections with Miriad (version 2) was estimated to be: HIV 3.7% (4/109; 95% CI 1.2% to 9.7%), HCV 42.2%

**Table 3** Accuracy and seropositivity data from Mumbai and Montreal

Category	Mumbai N=375	Montreal N=109
Sensitivity		
HIV	100% (95% CI 94.8% to 100%)	100% (95% CI 47.3% to 100%)
HBV	13.3% (95% CI 6.6% to 23.2%)	NA
HCV	50.0% (95% CI 1.3% to 98.7%)	80.4% (95% CI 66.1% to 90.6%)
Syphilis	86.1% (95% CI 70.5% to 95.3%)	100% (95% CI 22.4% to 100%)
Specificity		
HIV	99.7% (95% CI 98.3% to 99.9%)	100% (95% CI 97.2% to 100%)
HBV	99.3% (95% CI 97.6% to 99.9%)	100% (95% CI 97.3% to 100%)
HCV	99.7% (95% CI 98.5% to 99.9%)	85.3% (95% CI 73.8% to 93.0%)
Syphilis	85.2% (95% CI 80.9% to 88.8%)	98.1% (95% CI 93.3% to 99.8%)
Prevalence		
HIV	14.9% (95% CI 13.1% to 16.7%)	3.7% (95% CI 1.2% to 9.7%)
HBV	20.0% (95% CI 18.0% to 22.0%)	NA
HCV	0.5% (95% CI 0.2% to 0.9%)	42.2% (95% CI 32.9% to 52.0%)
Syphilis	9.9% (95% CI 8.4% to 11.4%)	1.8% (95% CI 0.3% to 7.1%)

HBV, hepatitis B; HCV, hepatitis C; NA, not available.

(46/109; 95% CI 32.9% to 52.0%) and syphilis 1.8% (2/109; 95% CI 0.3% to 7.1%). In terms of new infections, only one new case of HIV and one of syphilis were picked up with Miriad (version 2). At baseline, screening rates were 96.3% for HIV, 83.8% for HBV, 94.3% for HCV and 58.7% for syphilis.

In terms of preference, a majority of participants (97.2%, 106/109) preferred the multiplex test to conventional testing and would recommend it to others (99.1%, 108/109). In terms of turnaround time, about half (55.0%, 60/109) of the study participants wanted test results on the same day (TAT: 8 h), and only 19% (21/109) were willing to wait up to 1 week.

In terms of diagnostic performance, the sensitivities of Miriad (version 2) were: HIV 100% (95% CI 47.3% to 100%), HCV 80.4% (95% CI 66.1% to 90.6%) and syphilis 100% (95% CI 22.4% to 100%).

All participants had been vaccinated for HBV (as per Canadian guidelines); hence, no new infection was found, and HBV sensitivity could not be computed.

Specificities were as follows: HIV 100% (95% CI 97.2% to 100%), HCV 85.3% (95% CI 73.8% to 93.0%), syphilis 98.1% (95% CI 93.3% to 99.8%) and HBV 100% (95% CI 97.3% to 100%). Concordance was not computed for this component of the study because Miriad was performed by a single research nurse.

## DISCUSSION

The multiplexed POC based strategy was feasible to operationalise and preferred by a completely different set of populations in two different settings, with very different baseline rates of screening for HIV and co-infections, and varying levels of endemicity of these infections, and these explain the fact that detection of new infections differed in these two participants. IDUs in Montreal were heavily screened for HIV, HCV, HBV and vaccinated for HBV, while STD clinic attendees in Mumbai were heavily screened for HIV only and poorly vaccinated for HBV. With this strategy therefore in Mumbai, many new HIV and related co-infections were detected (ie, 75 HBV cases, 56 HIV and 37 syphilis), mainly because baseline screening rates in STD populations were very low in comparison to Montreal.

Regarding feasibility, completion rates computed were comparable and slightly higher in Montreal (92.4%) than in Mumbai (86.1%). This was mainly because some patients did not show up for the second visit in Mumbai. These participants cited loss of an additional working day's wages as a reason for not showing up. This implies that the number of visits required to collect test results may impact completion rates for POC-based strategies if they entail two visits, and more so in certain settings where laboratory results cannot be expedited for test result delivery. Getting actionable results is key to closing the POC continuum.<sup>21</sup> Therefore, before such tests are introduced in public health settings, it is necessary to envision a clear action plan—this action plan includes a

seamless integration of downstream confirmatory tests as per standard algorithms; integrating results from preliminary multiplexed POC devices will be essential to ensure rapid clinical action on the initial multiplexed screening result. This action could vary and may depend on the condition, the clinical management plan in the settings—it could include confirmatory testing or treatment referral or initiation. These action plans have profound consequences on the treatment and care cascade of poor vulnerable patient populations from resource restrained settings. If any of these steps are skipped, then the point of introducing a multiplexed test will be lost. Lastly, the feasibility of completion of a screening strategy could vary across population groups accessing it. Some groups may differ on their perceived risk for an infection (or co-infection), and this is an area of study, education and practice that needs to be explored further. In sum, a one size fits all strategy may not be the best approach for all subpopulations. Strategies may need to be modified according to the following variables or factors. These vary from patient-oriented outcomes such as preferences, lifestyles, circumstance, risk perception levels, vaccination history, past screening history, free testing versus co-pay, to health system level availability of confirmatory testing, treatment and clinical action plans and partner notification plans. Lastly, it is important to underscore an understanding of the downstream benefit of early screening for co-infections and immediate treatment or staging and awareness of a reduction in transmission risk to their partners, and children by patient participant communities. All these factors either alone or in combination will determine the success of multiplexed screening initiatives in countries and settings.

In terms of preference, high markings for multiplexed POC testing in both settings suggests that at-risk populations prefer the convenience of same day POC testing for several infections at once. However, in Mumbai, participants were willing to wait longer on average for their POC result than in Montreal. This could also be due to the fact that they had to travel long distances and take time off work to show up at a clinic. In Montreal, most of the participants did not mind showing up at this clinic. Again, delivery of the test result needs to be timed to patients' preferences and preparedness to receive them.

In terms of diagnostic performance of both versions of Miriad, the specificity was generally high for all four infections. In Montreal, version 2 showed an improved sensitivity for HCV (50.0–80.4%), and a perfect sensitivity (100%) for syphilis. The specificity and sensitivity parameters for each infection (combined) were comparable to the 95% CI reported for singleton POC tests.<sup>22–25</sup> Since the Miriad device used in this study was investigational and not in production, discussions of accuracy may be relevant for other similar biomarker-based devices in development. Similar diagnostic evaluations have been reported from the USA. In a study conducted by a group based at the US CDC,<sup>26</sup> the HIV/HCV test was evaluated

for performance and it performed well (sensitivity 89% and specificity 100%). In our test device, all the biomarkers for HBV, HIV and HCV detected antibodies, and for syphilis it detected antibodies to *Treponema* specific antigens.<sup>26</sup> In another study by Lochhead *et al*,<sup>27</sup> a fluorescence immunoassay was evaluated in known and controlled serum samples with good results. Our study is unique because, to the best of our knowledge, it was performed in a real-life setting; the aim of the study was to understand real-life challenges faced in the implementation of triplex/quadruplex multiplex assays and the impact they may have on the lives of patients. It also points to the need for health system priming before the introduction of these assays. Multiplex assays are being continuously improved for their accuracy—new studies released after completion of the trials will be assessed on an ongoing basis. The sensitivity of the HBV component in our Mumbai study was surprisingly low. This could perhaps be attributed to integrating Tp capture agents into a triple biomarker panel, and then needing to optimise the performance of the quadruple test. The key exploratory objective in both the studies is to move beyond accuracy towards outcomes that are patient centred. Such outcomes will have a more meaningful impact on the field of public health screening and diagnostics in particular.

In Montreal, nine participants were found to be Miriad 'positive' and HCV RNA 'negative'; thus, we also observed false-positive test results for HCV with a concomitant lower specificity, a phenomenon also reported in a recent study by Cha *et al*.<sup>28</sup> This interesting finding means that these patients were not infected with HCV when they were tested, but may have cleared the virus in the past. To confirm the antibody result following a negative RNA, the CDC recommends the performance of a second antibody test.<sup>29</sup> So it is reasonable to infer that the test result could have been a true positive with respect to HCV antibodies and that the person did not have an active infection. Further, some patients spontaneously clear infections, others clear it with treatment and yet others carry it to the next stage. Complexities in the interpretation of HCV and HBV results require the availability not only of reference standards in global settings but also of hepatologists to help interpret complex algorithms and treatment plans, especially in the setting of HIV co-infection. With the availability of newer and exciting treatment regimens for HCV, and cheaper and public vaccination programmes for HBV, addressing these issues is crucial to treatment staging and referral, while being highly pertinent in the roll-out of multiplexed screening initiatives.

In terms of implications of our study for research and practice, the performance of a multiplex strategy will be driven by many factors that act at multiple levels: population, patient, co-infections, device and health systems. First, population-level prevalence impacts pretest probability. In our study, while HCV prevalence was high in Montreal, HBV and syphilis prevalence were high in

Mumbai. Variable prevalence impacted our accuracy and seropositivity estimations. Second, macro patient-level factors impact accuracy. Past or partial treatment of co-infections influences current immune status. Furthermore, the role of one or more co-infections in impairing the diagnostic performance of multiplexed devices remains unknown. Immune suppression or modification and its impact on HCV estimation in Montreal could not be ruled out. Recent studies have shown that HCV antibodies can become more difficult to detect in the presence of HIV infection,<sup>30 31</sup> although we could not explore this issue in our study. Third, device-level factors such as the performance of each biomarker in a multiplexed POC device is expected to be comparable to the singleton POC tests, especially with respect to individual sensitivity parameters that may vary. Two of our published meta-analyses<sup>22 25</sup> showed that the sensitivity parameter for singleton POC tests for HBV and syphilis merited an improvement. By that comparison, the performance of the syphilis biomarker (100%) in version 2 of the device (used in Montreal) was surprisingly good, even with low numbers of infection. Similar issues were also raised by another study from the USA.<sup>32</sup> Lastly, the health system-level capacity and resources may impede the full benefit of multiplexing. The availability of high-quality, cost-efficient and reference standard tests and the best algorithms to use is always an issue. It is not enough to preliminarily screen and triage patients; confirmation of their results and treatment is equally important. Often, quality assured conventional reference standard tests for HBV, HCV and syphilis are not offered by public systems (as in Mumbai), and additional tests (ie, HCV RNA, HBV DNA, *Treponema pallidum* haemagglutination assay) in the algorithm inflated our overall costs of screening. In Canada, however, reference standard tests were available through the universal healthcare system, saving time and money for patients. In addition, in Mumbai, the lack of integrated linkages to treatment, referral and care for co-infections could also minimise the intended impact of multiplexed POC tests.

Therefore, for future practice and policy implications, multiplexed assays could be useful for preliminary screening and staging of concomitant infections in a single visit (ie, expedited triage tools), provided confirmatory testing, treatments are available and are not prohibitively expensive. In terms of the cost-effectiveness of this approach, although a POC test-based screening appears to be cost-effective, a broader analysis of prevalence and endemicity, price points of screening strategy with reference standards and treatments available, and manpower costs in different settings is urgently needed.

### Limitations

Study limitations included the use of a cross-sectional design, and convenience sampling of patients (generating a potential for possible volunteer bias and selection bias). Additionally, the wide CIs for sensitivities and a





low prevalence of co-infections in populations in Mumbai (for HCV) and in Montreal (for HBV) limited our accuracy estimations.

Device limitations included balancing device characteristics; while antibodies to one microbe may be efficiently detected using a running buffer of a specific pH or ionic strength, thus facilitating diagnosis, that running buffer may not be the ideal one to facilitate detection of antibodies to a second, third or fourth microbe. Manufacturers must make advances in this area to improve the performance of multiplexed assays. Phlebotomised venous blood was inputted into the MIRIAD device. Although it was intended to be a finger stick-based test, in some patients, in Montreal and drug users and CSWs, it was hard to collect the required amount of blood using a finger stick, so we decided to use a phlebotomised venous sample. We collected four vials of blood for reference standard testing, so a sample for a POC test was not difficult.

This first evaluation of a quadruple multiplexed biomarker-based assay offered insights pertinent to researchers, policymakers and funding agencies worldwide. It also offers insights into future product development, evaluation and envisioned integration of several such multiplexed initiatives that are being planned by public agencies. However, the potential impact of such initiatives will be much greater in settings where either the baseline screening rates are low, or the endemicity of co-infections is high. Therefore, background endemicity, prevalence, incidence of co-infections, value proposition of screening, cost benefit and cost-effectiveness analyses under limited assumptions and data are good starting points to help guide their implementation.

## CONCLUSION

To conclude, a multiplex strategy offering rapid simultaneous screening for HIV and related co-infections was feasible and preferred over conventional testing in diverse settings. It impacted the detection of new infections in a resource-limited setting and a population with low baseline rates of co-infection screening. Multiplex is a technology of the near future,<sup>33</sup> so envisioning its integration at various levels (ie, device, patient, health systems) today will determine its impact and success tomorrow.

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