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

Nitika Pant Pai,1,5 Rachita Dhurat,2 Martin Potter,3,4 Tarannum Behlim,5 Geneviève Landry,4 Caroline Vadnais,5 Camilla Rodrigues,6 Lawrence Joseph,7 Anjali Shetty6

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, 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.1–4 With a large share of cases going undetected,
the true burden of the co-infections can at best be wishful guesses. 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. 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. 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. 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. 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.

Diagnosis remains a critical step in infectious disease control, highlighting the need for timely targeted co-infections screening in at-risk populations. While syphilis facilitates HIV transmission, HIV/HCV and HIV/HBV co-infections facilitate disease progression to liver failure, cirrhosis or death. A timely diagnosis of HIV and HCV and HBV co-infections can minimise downstream adverse health effects, offset rapid disease progression, encourage care 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. 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. 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, 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. About 88% of the HIV-positive IDUs have a history of being infected with HCV. As for syphilis, the number of cases is on the rise since 2000, with 539 new cases reported in 2010. 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. 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
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
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.
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% (42.2% (95% CI 32.9% to 52.0%) and HBV 0.5% (95% CI 0.2% to 0.9%).

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

| Category          | Mumbai N=375                      | Montreal N=109                     |
|-------------------|-----------------------------------|-----------------------------------|
| Preliminary positive | 375 STD clinic attendees 118 IDUs | 118 IDUs                          |
| Gender            | 83.0% males 67.9% males            | 67.9% males                        |
| Age (mean)        | 31.2 years (19–63 years)           | 38.3 years (21–62 years)           |
| Previously tested for (baseline screening by conventional testing methods) |  |  |
| HIV               | 48.0% 96.3%                        | 96.3% 100%                        |
| HBV               | 1.6% 83.8%                         | 83.8% 100%                        |
| HCV               | 1.9% 94.3%                         | 94.3% 100%                        |
| Syphilis          | 2.7% 58.7%                         | 58.7% 100%                        |
| HBV, hepatitis B; HCV, hepatitis C; IDU, injection drug user; STD, sexually transmitted disease. |

**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.
These participants cited loss of an additional working time off work to show up at a 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 specificity 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.22–25 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,26 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 pallidum specific antigen. In another study by Lochhead et al., 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. 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. 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 coinfection. 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, 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 will be especially important with respect to individual sensitivity parameters that may vary. Two of our published meta-analyses 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. 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 haemaggulination 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 coinfections 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, so envisioning its integration at various levels (ie, device, patient, health systems) today will determine its impact and success tomorrow.

Acknowledgements The authors acknowledge the study staff at the following participating hospitals Hinduja Hospital, Mumbai, Sion Hospital, Mumbai, and CRAN clinics in Montreal, Canada. They thank the study participants at both sites for their time and patience. They also thank Sehar Manji and Jana Daher for their assistance in formatting and proofreading the manuscript.

Contributors NPP was involved in the conception, design, oversight of conduct, data integrity, writing and critical review. RD and MP were involved in the design, oversight of conduct, data integrity, writing and critical review. TB and CV were involved in the design, data integrity, writing and critical review. GL was involved in the design, oversight of conduct, writing and critical review. CR was involved in the conception, oversight of conduct, writing and critical review. LJ was involved in the design, oversight of conduct, data integrity, data analyses, writing and critical review. AS was involved in the conception, design, conduct, data integrity, writing and critical review.

Funding This study was funded by an operating grant from CIHR (HBF 103210) 2010. NPP also acknowledges receiving the CIHR New Investigator Award 2010. Grand Challenges Canada’s Star in Global Health Award 2013 and an operating grant from Bill and Melinda Gates Foundation (no OPP1061487).

Competing interests None.

Ethics approval McGill University Health Centre (lead institution), CRAN (collaborator in Canada), and P.D. Hinduja National Hospital and Medical Research Centre as well as Sion Hospital (collaborator in India).

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Data sets are available from the corresponding author on request (Excel format).

Open Access This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 3.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/3.0/.

REFERENCES
1. WHO. Global summary of the AIDS epidemic—December 2006. WHO, 2006 (cited 14 September 2009). http://www.who.int/hiv/mediacentre/02_Global_Summary_2006_EpiUpdate_eng.pdf
2. WHO U. Global AIDS Response progress reporting 2014. 2014.
3. sheet Wf. 2014. http://www.who.int/mediacentre/factsheets/fs164/en/.
(12 August 2014).
4. WHO. Hepatitis B fact sheet. 14 (12 August 2014). http://www.who.int/mediacentre/factsheets/fs204/en/.
5. WHO, UNAIDS, unicef. Global HIV/AIDS response: report update and health sector progress towards Universal Access, progress report 2011.
6. Klein MB, Willemsot P, Murphy T, et al. The impact of initial highly active antiretroviral therapy on future treatment sequences in HIV infection. AIDS 2004;18:1895–904.
7. Abuse CCoS. Injection Drug Users Overview. 2011 (updated 21 June 2013). http://www.ccsa.ca/EngTopics/Populations/IDU/Pages/InjectionDrugUsersOverview.aspx
8. Archibald CP, Jayaraman GC, Major C, et al. Estimating the size of hard-to-reach populations: a novel method using HIV testing data compared to other methods. AIDS 2001;15(Suppl 3):S41–8.
9. Challacombe L. The epidemiology of HIV in people who inject drugs in Canada. 2013.
10. UNAIDS. Ambitious treatment targets: writing the final chapter of the AIDS epidemic. Geneva, Switzerland: UNAIDS, 2014.
11. Ronald A, Kuyers J, Lukehart SA, et al. Excellence in sexually transmitted infection (STI) diagnostics: recognition of past successes and strategies for the future. Sex Transm Infect 2006;82(Suppl 5):v47–52.
12. De Cock KM, Bunneil R, Mermim J. Unfinished business—expanding HIV testing in developing countries. N Engl J Med 2006;354:440–2.
13. De Cock KM, Crowley SP, Lo YR, et al. Preventing HIV transmission with antiretrovirals. Bull World Health Organ 2009;87:488–488A.
14. Granich RM, Gilks CF, Dye C, et al. Universal voluntary HIV testing with immediate antiretroviral therapy as a strategy for elimination of HIV transmission: a mathematical model. Lancet 2009;373:48–57.
15. Weinhardt LS, Carey MP, Johnstone BT, et al. Effects of HIV counseling and testing on sexual risk behavior: a meta-analytic

Author affiliations
1Department of Medicine, McGill University, Montreal, Quebec, Canada
2Department of Dermatology, LTM Medical College, Mumbai, Maharashtra, India
3Chronic Viral Illness Service, Division of Infectious Disease, McGill University Health Centre, Montreal, Quebec, Canada
4Centre de recherche et d’ aide pour les narco manes (CRAN), Montreal, Quebec, Canada
5Division of Clinical Epidemiology, McGill University Health Centre, Montreal, Quebec, Canada
6Division of Microbiology, P.D. Hinduja National Hospital and Medical Research Centre, Mumbai, Maharashtra, India
7Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Montreal, Quebec, Canada
8Department of Medicine, McGill University, Montreal, Quebec, Canada
9Centre de recherche and d’ aide for narcomannes (CRAN), Montreal, Quebec, Canada
10Division of Clinical Epidemiology, McGill University Health Centre, Montreal, Quebec, Canada
11Division of Microbiology, P.D. Hinduja National Hospital and Medical Research Centre, Mumbai, Maharashtra, India
12Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Montreal, Quebec, Canada

Pai NP, et al. BMJ Open 2014;4:e005040. doi:10.1136/bmjopen-2014-005040
16. Klein MB, Lalonde RG, Suissa S. The impact of hepatitis C virus co-infection on HIV progression before and after highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* 2003;33:365–72.

17. Steinbrook R. HIV in India—a complex epidemic. *N Engl J Med* 2007;356:1089–93.

18. Public Health Agency of Canada. Summary: Estimates of HIV Prevalence and Incidence in Canada, 2011. 2011 (cited 1 December 2013). http://www.phac-aspc.gc.ca/aids-sida/publication/surnreport/estimat2011-eng.php.

19. LeBlanc M. What the syph is going on? Responding to syphilis outbreaks in Canada. 2013 (21 January 2014). http://www.catie.ca/en/printpdf/pi/spring-2013/what-syph-going-responding-syphilis-outbreaks-canada.

20. Kitahata MM, Gange SJ, Abraham AG, *et al.* Effect of early versus deferred antiretroviral therapy for HIV on survival. *N Engl J Med* 2009;360:1815–26.

21. Pai NP, Vadnais C, Denkinger C, *et al.* Point-of-care testing for infectious diseases: diversity, complexity, and barriers in low- and middle-income countries. *PLoS Med* 2012;9:e1001306.

22. Jafari Y, Peeling RW, Shivkumar S, *et al.* Are Treponema pallidum specific rapid and point-of-care tests for syphilis accurate enough for screening in resource limited settings? Evidence from a meta-analysis. *PLoS ONE* 2013;8:e54695.

23. Pai NP, Balram B, Shivkumar S, *et al.* Head-to-head comparison of accuracy of a rapid point-of-care HIV test with oral versus whole-blood specimens: a systematic review and meta-analysis. *Lancet Infect Dis* 2012;12:375–80.

24. Shivkumar S, Peeling RW, Jafari Y, *et al.* Accuracy of rapid and point-of-care screening tests for hepatitis C: a systematic review and meta-analysis. *Ann Intern Med* 2012;157:558–66.

25. Shivkumar S, Peeling R, Jafari Y, *et al.* Rapid point-of-care first-line screening tests for hepatitis B infection: a meta-analysis of diagnostic accuracy (1980–2010). *Am J Gastroenterol* 2012;107:1306–13.

26. Smith BD, Drobeniuc J, Jewett A, *et al.* Evaluation of three rapid screening assays for detection of antibodies to hepatitis C virus. *J Infect Dis* 2011;204:825–31.

27. Lochhead MJ, Todorof K, Delaney M, *et al.* Rapid multiplexed immunoassay for simultaneous serodiagnosis of HIV-1 and coinfections. *J Clin Microbiol* 2011;49:3584–90.

28. Cha YJ, Park O, Kang ES, *et al.* Performance evaluation of the OraQuick hepatitis C virus rapid antibody test. *Ann Lab Med* 2013;33:184–9.

29. CDC. Testing for HCV Infection: An Update of Guidance for Clinicians and Laboratorians. 2013.

30. French AL, Operskalski E, Peters M, *et al.* Isolated hepatitis B core antibody is associated with HIV and ongoing but not resolved hepatitis C virus infection in a cohort of US women. *J Infect Dis* 2007;195:1437–42.

31. Witt MD, Lewis RJ, Rieg G, *et al.* Predictors of the isolated hepatitis B core antibody pattern in HIV-infected and -uninfected men in the multicenter AIDS cohort study. *Clin Infect Dis* 2013;56:606–12.

32. Waldeisen JR, Wang T, Mitra D, *et al.* A real-time PCR antibiogram for drug-resistant sepsis. *PLoS ONE* 2011;6:e28528.

33. Pai NP, Pai M. Point-of-care diagnostics for HIV and tuberculosis: landscape, pipeline, and unmet needs. *Discov Med* 2012;13:35–45.
Supplement Figures:

Figure S1: Miriad (TM) multiplexed investigational point-of-care test device
Figure S2: Flow of participants in Mumbai

All consenting patients with suspected HIV, HBV, HCV or Syphilis infection (N=500)

Dropped out and did not provide consent after the study procedure was explained (N=125)

Miriad™ test performed by physician and phlebotomist (N=375)

Blood sample collected for confirmatory testing (N=375)

Post-test combined counseling with key information on four infections, prevention and benefits of early diagnosis (N=375)

Exit interview through administration of semi-structured questionnaire; follow-up visit arranged to disclose confirmatory test results (N=375)

Patients lost to follow up – did not complete their second visit (N=52)

Confirmatory test result communicated (N=323)
Figure S3: Flow of participants in Montreal

Number of participants approached (N=155)

All consenting patients with suspected HIV, HBV, HCV or Syphilis infection (N=118)

Miriad\textsuperscript{TM} test performed by research nurse (N=109)

Blood sample collected for confirmatory testing (N=109)

Post-test combined counseling with key information on four infections, prevention and benefits of early diagnosis (N=109)

Exit interview through administration of semi-structured questionnaire; follow-up visit arranged to disclose confirmatory test results (N=109)

Confirmatory test result communicated (N=109)