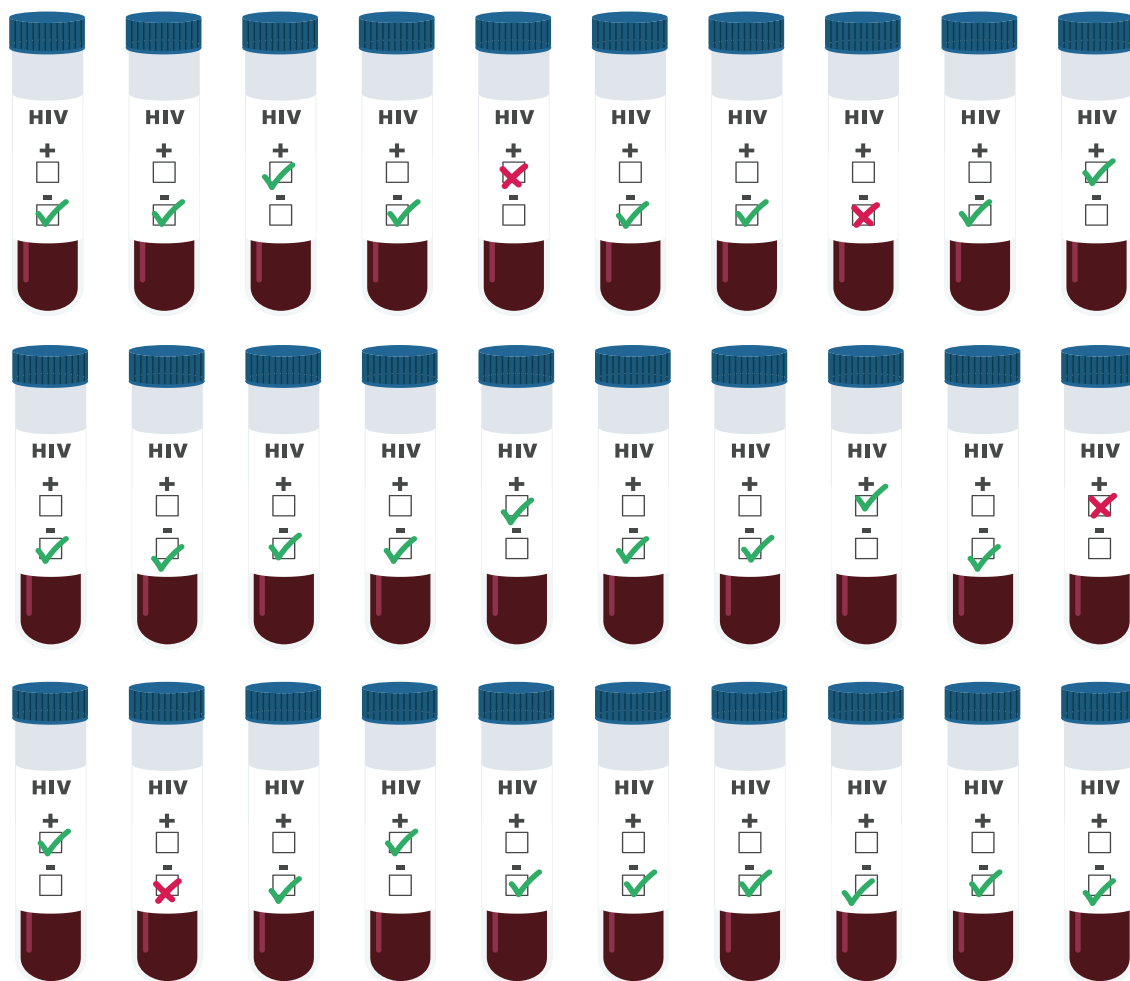


Programmatic and public health implications of misdiagnosis of HIV

Guest Editors: Shona Dalal, Cheryl C. Johnson, Miriam Taegtmeier
Supplement Editors: Marlène Bras, Elisa de Castro Alvarez



Support

The publication of this supplement was supported through the U.S. Agency for International Development (USAID), with support from the U.S. President's Emergency Plan for AIDS Relief, the Brocher Foundation and the World Health Organization. The content of this supplement is solely the responsibility of the authors and does not necessarily represent the official views of the United States Government or the World Health Organization.



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Editorial

A public health approach to addressing and preventing misdiagnosis in the scale-up of HIV rapid testing programmes

Cheryl C. Johnson^{1,2,5}, Shona Dalal¹, Rachel Baggaley¹ and Miriam Taegtmeier³

⁵**Corresponding author:** Cheryl C. Johnson, Department of HIV, World Health Organization, 20 Avenue Appia, Geneva, 1201, Switzerland. (Johnsonc@who.int)

Keywords: HIV; diagnostic; rapid diagnostic test; test; quality; misdiagnosis; misclassification

Received 26 June 2017; Accepted 03 July 2017; Published 29 August 2017

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Introduction

The global impact of the scale-up HIV testing and treatment has been impressive. In 2015, approximately 60% of people with HIV worldwide were aware of their status [1]. As a result by the end of 2015, 17 million people with HIV were on treatment, and global treatment coverage reached 46% [1]. HIV testing and treatment have reduced AIDS-related deaths by 43% since 2003 [1,2]. In order to further increase impact and improve health outcomes, in 2016 the World Health Organization (WHO) recommended antiretroviral therapy (ART) for all people with HIV regardless of disease status [3]. These calls to continue scale-up of testing and treatment and to achieve the United Nation's (UN) "90-90-90" targets remain a global priority. Achieving the "first 90" by reaching people with HIV who have yet to be diagnosed, and linking them to treatment as early as possible, is a critical first step.

Degrees of uncertainty exist with all medical testing and diagnoses; in the field of HIV, advances in diagnostic test technology have made testing accurate and reliable. WHO prequalified HIV rapid diagnostic tests all have a sensitivity of $\geq 99\%$ and specificity $\geq 98\%$ and are accurate when used correctly in a validated national algorithm. A large number of tests are conducted every year. Although a degree of error and misdiagnosis can be expected, very few cases of false negative and false positive diagnoses have been reported [4–12]. This lack of reporting on testing error and misdiagnoses is not unique to HIV [13–16]. Publication bias and concerns about programme reputation may have contributed to low reporting of misdiagnosis and limit the open discussion required to address errors systematically [16].

To further investigate diagnostic error, determine common causes, and identify potential ways to address misdiagnosis, particularly in resource-limited settings, WHO, Liverpool School of Tropical Medicine and Médecins Sans Frontières (MSF) held a symposium to address the social, public health, human rights, ethical and legal implications

of misdiagnosis of HIV status [17]. This special issue of *the Journal of the International AIDS Society* follows this symposium by focusing on the individual and public health implications of HIV misdiagnosis.

Is HIV misdiagnosis a "real" problem?

Data from a systematic review of 64 studies (most studies identified were conducted in Africa and other resource-limited settings) are included in this special issue and summarize the magnitude of misdiagnosis in these contexts. The review suggests that on average 0.4% (interquartile range (IQR): 0–3.9%) of diagnoses primarily among adults are false negative and 3.1% (IQR: 0.4–5.2%) are false positive [18]. Among people diagnosed with HIV who were enrolled in care and/or on ART, between 0.1% and 6.6% of patients were reported to be truly HIV negative and had been misdiagnosed [18]. The diagnostic errors identified were largely related to human error [18]. Although reported levels of misdiagnosis are low, if current estimates are accurate [18,19], the large volume of tests conducted each year - over 150 million tests in low- and middle-income countries in 2014 alone, 3 million of which were HIV positive [19] - could result in the misdiagnosis of up to 93,000 people per year if left unaddressed.

What factors and processes contribute to misdiagnosis using rapid tests?

HIV misdiagnoses and testing errors are unlikely to be the result of a single cause or underlying factor. Diagnostic errors can occur across multiple steps within the HIV testing continuum, starting from national policy and training, through the supply chain, initial testing and the delivery of a diagnosis, including retesting patients prior to ART initiation as well as inadvertently retesting patients on ART who may re-present for testing erroneously (Figure 1).

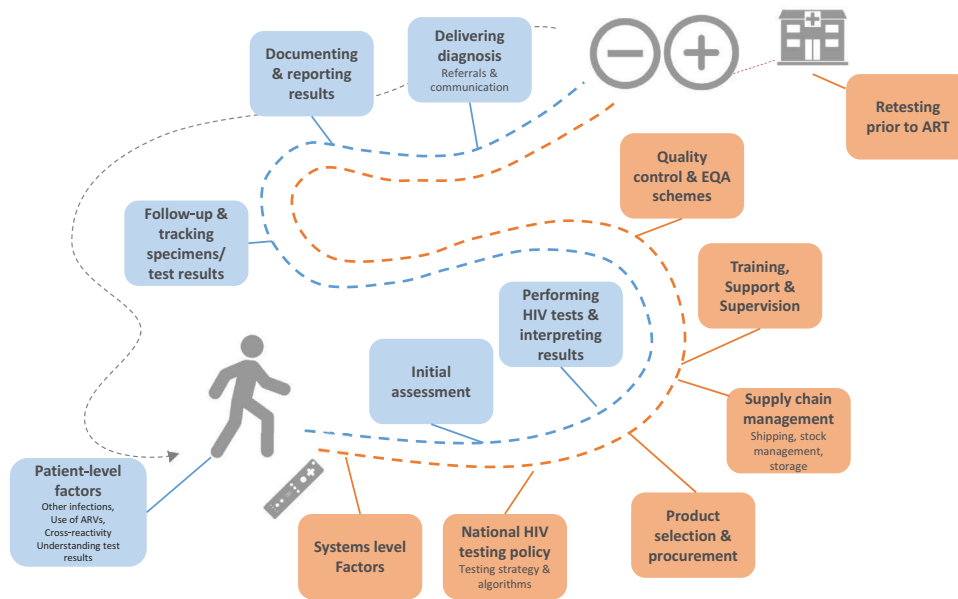


Figure 1. HIV testing continuum and the patient, provider, facility and system-level dimensions of where diagnostic errors and HIV misdiagnosis can occur [20].

The review by Johnson et al. [18] highlighted that the use of suboptimal testing algorithms was a common cause of misdiagnoses in studies reviewed. In this issue, Bock et al. report that by using a first-line assay with poor sensitivity in South Africa, the resulting programmatic sensitivity was as low as 45% (95% confidence interval: 23–48) [21]. Another known contributor to misdiagnosis is the use of a tiebreaker test to rule in HIV infection after discrepant test results, which can cause a high proportion of false positive diagnoses. Although this strategy is known to be inferior to providing patients with an inconclusive status and requesting them to retest in 14 days, many programmes continue to use a tiebreaker out of convenience, the desire to make an immediate diagnosis so that ART can be initiated and concerns that clients will be lost to follow-up [22].

User and clerical errors at testing sites are also a factor as reported by Khan et al. in this issue [23]. In their study, nearly all of the misdiagnosed patients unnecessarily placed on ART reported that they had at least one HIV-negative test result before they were started on treatment; additionally, two other patients reported that they were never shown their test results despite being given an HIV-positive diagnosis. Authors suggest these cases of misdiagnosis were likely due to administrative error, user error and clients' circumstantial belief that they were HIV positive [23]. Thus, it is likely that these cases of misdiagnosis could have been prevented if health workers had a clear understanding of how to communicate uncertainty of test results and if procedures for addressing potential misdiagnoses were in place.

Other reported factors, related specifically to false negative diagnoses, included early/acute HIV infection [24] and testing among people on ART (e.g. people who retest without disclosing their ART use) [18]. According to Kufa

et al., false negative diagnoses were associated with a reactive HIV-1 Limiting Antigen enzyme immunoassay (LAG EIA) test result (i.e. a marker of early/acute infection), individuals reporting an HIV-positive status and those reporting ART use [24]. Patients on ART who retest may have low levels of detectable HIV antibodies. Two reports in this issue found that 8.5–44% of false negative diagnoses were among people on ART who were retested [21,25]. According to Olaru et al. [25], which sought to investigate the impact of ART on test performance, 8.5% of children with HIV on ART had a false negative test result when using an oral fluid-based rapid diagnostic test (RDT), and those who had been on ART longer and who had higher CD4 counts were more likely to have a negative test.

Understanding the factors contributing to misdiagnosis across specific contexts is critical to developing a public health approach that will be effective in both addressing and preventing misdiagnosis in the scale-up of HIV rapid testing programmes.

What are the consequences and costs of misdiagnosis?

The importance, and possible consequences, of misdiagnosis should not be underestimated. On an individual level, false positive diagnoses can lead to unnecessary financial expenses, clinical visits and treatment initiation causing physical, emotional and psychosocial harm [12,23,26]. According to an MSF study, many of those misdiagnosed were not identified and re-diagnosed as HIV negative for at least a year during which 10 people were placed on ART: six for treatment, and four (two mother–baby pairs) to prevent

vertical transmission [12]. While the majority of those misdiagnosed were pleased to learn they were HIV negative, several were reportedly overwhelmed by the news as their HIV-positive diagnosis had disrupted their lives through stigma, broken relationships and divorce [12]. Additionally, missed opportunities to diagnose HIV due to false negative results continues to cause delays in the initiation of life-saving treatment and contributes to both on-going HIV transmission and HIV-related morbidity in adults, and as reported by Technau et al. in this issue, amongst HIV-exposed infants with inconclusive results [27].

The potential financial and economic cost of false misdiagnoses is likely to be high. False positive diagnoses, as reported in South Africa by Hsiao and colleagues in this issue [28], lead to unnecessary ART costs, even with $\leq 1\%$ misdiagnoses. Mathematical modelling suggests programmes which do not retest people with an HIV positive diagnosis prior to initiating treatment could spend between US\$58,000 and US\$225,000 in unnecessary ART costs per year for both low (1%) and high (10%) HIV prevalence settings [29]. Since there is currently no available HIV testing technology validated for testing people on ART, alternative strategies to determine a patient's true HIV status after they start treatment require the testing of viral reservoirs [30–32]. These strategies are not only complex, unfeasible in many settings and costly, but ill-advised as they could be potentially harmful to patients. Additionally, as many patients are now offered treatment immediately after diagnosis, the occurrence of seroreversion among patients on treatment may become more common, especially among infants and children [25]. Understanding the implications and the best practices to address retesting among people on ART, as well as the potential implications for retesting people on pre-exposure prophylaxis, is an area needing further research that can guide the implementation of practical solutions.

From a public health perspective, misdiagnoses in the context of HIV surveillance may result in under- or over-estimations of HIV prevalence and may have particular implications when programme data from RDT are used [33,34]. False negative diagnoses could also lead to further HIV transmission by providing a false sense of security. As many as 70% of new HIV transmissions may be attributable to undiagnosed HIV infection [35], with early/acute infection contributing to 10–50% of new HIV transmissions [36]. Furthermore, misdiagnoses may also undermine public trust in test results as well as trust in health services. Such distrust can be detrimental, as it can be a barrier preventing and delaying individuals from accessing services [37], potentially exacerbating gaps in HIV testing, prevention and treatment coverage.

Are there additional challenges to addressing misdiagnosis in infants and children?

Sacks et al. [38] note a number of key differences affecting the interpretation and management of test results in infants and children such as vertical transmission dynamics and the natural history and decay of maternal antibodies.

The consequences of delayed, false negative and false positive diagnoses, while serious for all ages, are more so for children. Inconclusive results delayed delivery of a final HIV-positive diagnosis; and 17% of infants with HIV with an inconclusive diagnosis died [27]. Olaru et al. note that some children who start ART early in life never develop HIV antibodies to establish a definitive HIV diagnosis [25], making cases where HIV-negative infants are unnecessarily placed on treatment even more challenging to resolve [38]. With these challenges in mind, in order to address and minimize misdiagnosis in infants and children, it is an urgent priority to retain all HIV-exposed infants and children with HIV-negative or inconclusive test results in care until a final diagnosis is ascertained after completion of breastfeeding and for testing to verify the HIV status of any child who has an initial nucleic acid test with detectable results immediately [38].

How should HIV misdiagnosis be prevented and addressed?

A combination of policy and programmatic approaches will be needed to address and prevent misdiagnoses. As outlined by Singh and Sittig in the “Safer Dx framework” (Figure 2) [39], preventing error and misdiagnosis will require a variety of stakeholders including researchers, health workers, policymakers, programme managers, implementing partners, civil society and patient advocates to develop and implement strategies and tools for measuring and monitoring diagnostic error, as well as to provide feedback and learning to inform the implementation of interventions that minimize misdiagnoses, improve testing quality and result in improved patient outcomes.

First, ensuring that appropriate and quality-assured tests are selected and procured based on a proven testing strategy and validated testing algorithm is critical. A 2015 policy review suggested that fewer than 20% of national HIV testing strategies were in line with WHO recommendations [40]. Revising these strategies to ensure that a sensitive first-line assay and referral of discrepant results for retesting at 14 days are used instead of using a tiebreaker will have a significant impact. Conducting or utilizing findings from testing algorithm validation studies is a key way programmes can reduce the risk of misdiagnosis. Although additional resources may be needed to validate algorithms and replace the use of a tiebreaker test with active follow-up and testing of patients with inconclusive results, this is likely to be a better investment than continuing to deliver potentially incorrect test results which may lead to the unnecessary ART initiation.

Second, retesting prior to ART initiation should be implemented as a routine service and considered the standard of care [17,41]. Despite some concerns about potential costs and feasibility, it is cost-effective [28,29], and can improve testing quality and reduce misdiagnosis. Programmatic reports from Malawi show that since implementing retesting prior to ART, together with retraining testers and introducing new guidelines on supervision, the proportion of patients misdiagnosed has decreased from approximately 7% in 2014 to 1% in 2016 [42].

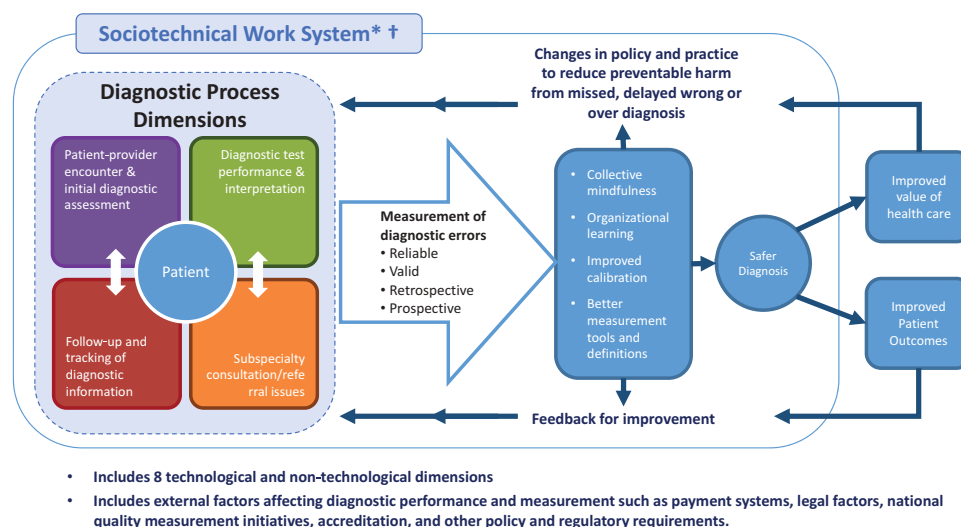


Figure 2. Safer Dx framework, adapted from Singh and Sittig 2015 [39].

Third, quality assurance systems are essential to HIV testing services. Training, support and supervision are key for all HIV testing providers, as well as routine external quality assessment schemes which help identify where problems may be occurring and which HIV testing providers and sites might benefit most from additional training and support. Standardized testing registers and logbooks improve reporting systems and, when maintained, can help programmes quickly identify and assess emerging quality issues and take corrective action. Electronic record systems can accelerate the identification of errors and misdiagnoses by quickly gathering and assessing patient-level information and tracking and following-up of those with characteristics linked to misdiagnosis [43]. The assignment of a unique patient identifier so that all of the testing results for the one client can be followed and evaluated over time is critical to identifying potential misdiagnosis.

Many of the studies included in this special issue are examples of how investigating quality issues can lead to improvements in testing services and how combining quality assurance systems with scale-up can mean that increasing quantity does not necessarily compromise quality. Nguyen and colleagues [44] were able to simultaneously scale-up HIV testing for key populations through community-based services and also prevent misdiagnosis by using a valid testing algorithm and quality assessment tools and systems. Bock and colleagues demonstrate that through assessing testing quality and identifying serious testing issues, by following up and implementing retraining, additional supervision, the use of a second reader for RDT results and retesting prior to ART, testing quality improved and errors became infrequent [21].

Can we have quantity and quality?

Correct HIV test results are one of the WHO “5Cs” and a guiding principle to the delivery of HIV testing services

worldwide. Achieving the UN 90-90-90 targets is key to the global public health agenda; however, achieving these goals while also meeting quality testing standards has proven difficult. Continued expansion of HIV testing services and treatment has tremendous individual and public health benefits, but must include accurate diagnosis. Now that ART will be offered to all people with HIV immediately after diagnosis, preventing and addressing misdiagnosis is of paramount importance. Every effort to prevent and address misdiagnosis if and when it occurs must be made alongside the scale-up of HIV testing services.

Communicating and coping with uncertainty in any health-related test results is difficult for healthcare providers and patients alike. On occasion, it may not be possible to deliver an HIV diagnosis on the same day, and further testing after a period of time will be needed. This message must be understood and conveyed by testing providers to their clients. Developing community messaging around the limitations of testing in certain contexts, despite their high accuracy and reliability, may be beneficial. In particular, messaging around the possibility that some clients may not be able to receive a same day diagnosis and returning for test results will be needed. Furthermore, although uncertainties may occur, misdiagnoses are mostly preventable through quality systems, appropriate algorithm use, retesting prior to ART initiation and follow-up procedures to correct discrepancies should they arise.

Current research demonstrating the benefits of immediate ART, for the individual and to prevent transmission, has led activists, national governments, international donors and the non-governmental community to fund and implement unprecedented efforts to provide treatment for everyone with HIV. We now need the same level of activism and global commitment to insist on accuracy of HIV testing.

Authors' affiliations

¹Department of HIV, World Health Organization, Geneva, Switzerland;
²London School of Hygiene and Tropical Medicine, Department of Clinical Research, London, UK; ³Liverpool School of Tropical Medicine, Liverpool, UK

Competing interests

We declare no competing interests. The authors alone are responsible for the views expressed in this article and they do not necessarily represent the views, decisions or policies of the institutions with which they are affiliated.

Authors' contribution

CJ and MT conceived of the special issue. CJ, SD and MT reviewed all articles in the special issue and formulated the editorial. CJ, SD and MT all drafted and developed the editorial. All authors have read and approved the final version.

Acknowledgements

We would like to thank Marlene Bras, Elisa de Castro Alvarez, Nathan Ford, Elizabeth Marum, Caitlin Payne, Victoria Watson, Russell Dacombe, Anita Sands, Meg Doherty and Lara Vojnov who provided input into this editorial and their support in coordinating the special issue on misdiagnosis of HIV, as well as all the co-organizers and participants of the misdiagnosis symposium and all the authors and peer reviewers who contributed to this special issue.

Funding

The publication of this supplement was supported through the U.S. Agency for International Development (USAID), with support from the U.S. President's Emergency Plan for AIDS Relief, the Brocher Foundation and the World Health Organization. The content of this supplement is solely the responsibility of the authors and does not necessarily represent the official views of the USA Government or the World Health Organization.

Disclaimer

Funders had no role in developing the editorial or the special issue.

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Review article

To err is human, to correct is public health: a systematic review examining poor quality testing and misdiagnosis of HIV status

Cheryl C. Johnson^{1,2§}, Virginia Fonner³, Anita Sands⁴, Nathan Ford¹, Carla Mahklouf Obermeyer⁵, Sharon Tsui⁶, Vincent Wong⁷ and Rachel Baggaley¹

[§]**Corresponding author:** Cheryl C. Johnson, Department of HIV, World Health Organization, 20 Avenue Appia, Geneva, 1201, Switzerland. (Johnsonc@who.int)

Abstract

Introduction: In accordance with global testing and treatment targets, many countries are seeking ways to reach the “90-90-90” goals, starting with diagnosing 90% of all people with HIV. Quality HIV testing services are needed to enable people with HIV to be diagnosed and linked to treatment as early as possible. It is essential that opportunities to reach people with undiagnosed HIV are not missed, diagnoses are correct and HIV-negative individuals are not inadvertently initiated on life-long treatment. We conducted this systematic review to assess the magnitude of misdiagnosis and to describe poor HIV testing practices using rapid diagnostic tests.

Methods: We systematically searched peer-reviewed articles, abstracts and grey literature published from 1 January 1990 to 19 April 2017. Studies were included if they used at least two rapid diagnostic tests and reported on HIV misdiagnosis, factors related to potential misdiagnosis or described quality issues and errors related to HIV testing.

Results: Sixty-four studies were included in this review. A small proportion of false positive (median 3.1%, interquartile range (IQR): 0.4-5.2%) and false negative (median: 0.4%, IQR: 0-3.9%) diagnoses were identified. Suboptimal testing strategies were the most common factor in studies reporting misdiagnoses, particularly false positive diagnoses due to using a “tiebreaker” test to resolve discrepant test results. A substantial proportion of false negative diagnoses were related to retesting among people on antiretroviral therapy.

Conclusions: HIV testing errors and poor practices, particularly those resulting in false positive or false negative diagnoses, do occur but are preventable. Efforts to accelerate HIV diagnosis and linkage to treatment should be complemented by efforts to improve the quality of HIV testing services and strengthen the quality management systems, particularly the use of validated testing algorithms and strategies, retesting people diagnosed with HIV before initiating treatment and providing clear messages to people with HIV on treatment on the risk of a “false negative” test result.

Keywords: HIV; HIV testing; misdiagnosis; misclassification; diagnostic error; false positive; healthcare; patient safety

To access the supplementary material to this article please see [Supplementary Files](#) under Article Tools online.

Received 16 December 2016; **Accepted** 07 March 2017; **Published** 29 August 2017

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Introduction

In the last decade, HIV testing services have been scaled-up substantially. In 2005, it was estimated that only 12% of people who wanted an HIV test were able to access testing; and that only 10% of people with HIV in Africa knew their status [1]. In contrast, between 2010 and 2014, more than 600 million people in 122 low- and middle-income countries received HIV testing [2], and as of 2015, approximately 60% of people with HIV were aware of their status [3]. Such scale-up has been possible through the expansion of provider-initiated testing and counselling and community-based testing

programmes, which have routinized HIV testing and extended services to many people.

Rapid diagnostic tests (RDTs) have been instrumental to the scale-up of HIV testing, particularly in resource-limited settings where access to laboratory services is poor. RDTs have been shown to be highly accurate and can often provide a same-day diagnosis when used within a validated testing strategy (i.e. the order in which the tests are performed) and algorithm (i.e. the exact tests used within the testing strategy) according to high ($\geq 5\%$) and low HIV prevalence ($< 5\%$), as recommended by the World Health Organization

(WHO) [4–6]. Recent reports, however, have shown that HIV testing is not always conducted appropriately [7,8], and in some countries, quality systems have not kept pace with testing scale-up. According to a review of national HIV testing policies, less than 20% of testing strategies were consistent with WHO guidance, and only two included recommendations on retesting prior to the initiation of antiretroviral therapy (ART) [9]. In some cases, poor-quality testing has resulted in incorrect test results and the misdiagnosis of HIV status [10–14].

HIV misdiagnosis refers to any testing event where a diagnosis is missed, inappropriately delayed or incorrect (either false positive or false negative) [15]. Poor-quality HIV testing and misdiagnosis have negative consequences for individuals, families, communities, health workers and health services. False negative diagnoses represent missed opportunities to identify an HIV infection and link people to early treatment. False positive diagnoses may cause social and emotional harm and create mistrust of health workers and the test results they deliver. Without addressing HIV testing quality, new guidance offering same-day treatment to all people diagnosed with HIV [16] could lead to inappropriate ART initiation [11]. Once individuals are on treatment, because ART reduces antibody production and can cause seroreversion, for example, false negative test results, determining a person's true HIV status can be especially challenging [17,18].

We conducted this systematic review to assess the magnitude of misdiagnosis and to identify and describe poor HIV testing practices using RDTs, including those which may have led to incorrect test results and misdiagnosis.

Methods

We systematically searched for peer-reviewed articles published from 1 January 1990 to 19 April 2017 using a predefined search strategy in the following electronic databases: PubMed, CINAHL and EMBASE. All conferences of the International AIDS Society were searched from July 2001 through July 2016; the most recent Conference on Retroviruses and Opportunistic Infections (2014–2017) database were searched because past conference abstracts were unavailable. Conferences of the African Society of Laboratory Medicine (ASLM) were searched 2012–2016, as well as the ASLM website and other key global health websites (see supplementary information). We searched reference lists to identify additional literature. This process was repeated until no new citations were identified. Experts were also contacted to identify additional reports. No geographic restrictions were placed on the search, but the review was limited to studies published in English.

Studies were eligible if they used at least two RDTs and reported on HIV misdiagnosis, factors related to potential misdiagnosis or described quality issues and related to HIV testing error.

Initial titles were screened by one investigator (VF) to determine eligibility. A second and a third screening was then carried out (VF, ST and CJ). All differences were resolved through consensus. Data from all sources were extracted and placed into standardized forms and verified

in duplicate (VF and ST). CJ and NF assessed study quality (see supplementary data).

Potential factors relating to misdiagnosis were extracted from studies using defined categories: (a) clerical error (error in documenting and reporting information essential to a correct status); (b) user error (operator error collecting specimen, performing an HIV RDT or interpreting the result); (c) suboptimal testing strategy (errors related to the order in which specific RDTs are used, also known as a testing strategy); (d) poor management and supervision (lack of active quality management systems); (e) weak reactive results (faint lines appearing on test strips); and (f) additional factors including cross-reactivity, acute/early infection and testing among people on ART.

Other summary measures included: misdiagnosis rates (total number of false positive diagnoses reported over the total number of HIV-positive tests retested and reported using a specific testing algorithm and the total number of false negative diagnoses reported over the number of HIV-negative tests retested and reported using a specific testing algorithm). For studies exclusively among people diagnosed with HIV, reporting on false positive statuses, the total study population was used as the denominator.

For each study, rates of diagnostic error and misdiagnosis and corresponding 95% confidence intervals (CIs) were calculated, using Wilson's approach, and this was displayed graphically using forest plots [19–21]. All statistical analyses were conducted in STATA v13.0.

Results

Sixty-four studies reporting on misdiagnosis of HIV and factors potentially related to misdiagnosis were included in this review (Figure 1 and Table 1).

Most studies were carried out in Africa ($n = 48$) [5,7,10–14,16,22–25,29,30,32–34,36,37,39,41,43,44,46,47,49,51,52,54–59,61–65,67–72,74,75], followed by in the Americas ($n = 7$) [28,31,42,50,53,60,66], Asia ($n = 4$) [8,35,45,73] and Europe ($n = 1$) [48]. There were also four multi-country/regional studies [26,27,38,40]. Samples varied by size and unit of measurement, including clients ($n = 38$ studies, range: 303,010 to 1 clients), specimens ($n = 15$ studies, range: 9419 to 16 specimens), health workers performing HIV tests ($n = 5$ studies, range: 3835 to 39 personnel) and sites where HIV testing was performed ($n = 12$ studies, range: 602 to 4 sites). Nine studies reported more than one unit of measure, and three studies did not specify sample size (see supplementary information). The majority of studies occurred in a facility-based setting; studies carried out in community settings included the workplace ($n = 1$) [57], home-based testing ($n = 2$) [14,39] and a mobile setting ($n = 1$) [32].

Factors related to the quality of HIV testing and potential misdiagnosis

Several factors, including HIV testing errors, were reported frequently ($n = 131$ times) across all included studies (see Table 2).

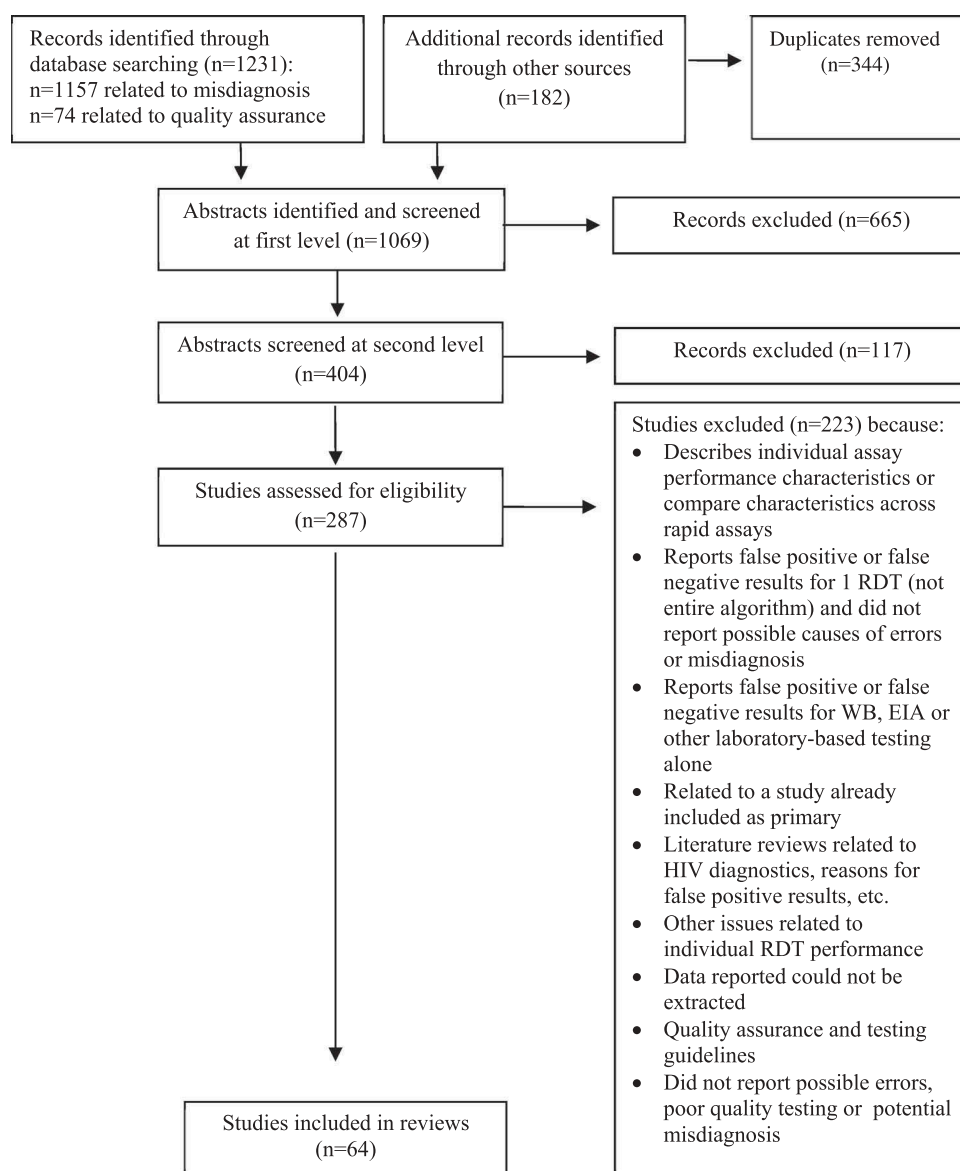


Figure 1. Study selection process.

RDT: rapid diagnostic test; WB: Western blot; EIA: enzyme immunoassay.

Thirty-seven studies reported using a suboptimal testing strategy that differed from the WHO recommendations [5,8,11–14,16,22–30,32–34,36–39,42–44,49,51,53,59,62,64–66,68,72,75]. Suboptimal testing strategies included using a highly specific first-line test and highly sensitive second-line test [14,33,39,55], using a single RDT for HIV-positive diagnoses [11,66,72], using a high prevalence testing strategy in a low prevalence setting [16,49], using a parallel testing algorithms and a tiebreaker testing strategy (where a third assay is used to resolve discrepant test results and rule in HIV infection) [5,12,13,16,24,25,27–30,32,34,36,37,68].

User errors, incorrectly performing the test procedure or incorrectly interpreting results, defined as human errors, were

reported in 25 studies [7,8,11,14,26–28,31,33,34,37,40,42,46,52,54,57,60,65–68,70,72,73]. Errors identified included users having difficulty with specimen collection [14,28,68], performing RDTs [31,73], interpreting test results [10,24,27,30,32,40,42,48,62,65,66,74], reading test results too early [7] and not using the correct reagents/buffer [7].

Twenty-one studies reported inadequate management and supervision [7,8,11,26,27,31,41,43,46,52,59,62,64–67,69,71,72,74]. Of these, 10 studies reported issues with management of supplies [7,11,26,27,62,64,67,69,72,74], including stock-outs [7,26,62,64,67,69], the use of damaged or expired RDTs [26,27,64,67] and inappropriate RDTs (i.e. syphilis RDTs) for HIV testing [72]. Other factors related to poor management and supervision included testing within the

Table 1. Classification of included studies (n = 64)

| Category | Study | Location |
|--|--------------------------------------|--|
| Potential HIV misdiagnosis and related factors | Aghokeng et al. [22] | Cameroon |
| | Baltazar et al. [23] | Mozambique |
| | Baveewo et al. [24] | Uganda |
| | Bock et al. [14] | South Africa and Zambia |
| | Boeras et al. [25] | Zambia and Rwanda |
| | CDC [26] | Low- and low–middle-income countries (not specified) |
| | Crucitti et al. [27] | Benin, India, South Africa, Uganda and India |
| | da Costa et al. [28] | Brazil |
| | Eller et al. [29] | Uganda |
| | Fogel et al. [23] | Multiple countries in Africa |
| | Galiwango et al. [30] | Cameroon |
| | Granade et al. [31] | USA |
| | Gray et al. [32] | Uganda |
| | Hsiao et al. [33] | South Africa |
| | Jentsch et al. [34] | South Africa, Tanzania, Uganda and Zambia |
| | Kanal et al. [35] | Cambodia |
| | Karugaba et al. [36] | Uganda |
| | Khan et al. [37] | Swaziland |
| | Klarkowski et al. [38] | Central Africa Republic, Congo, DRC, Ethiopia, Haiti, India, Cote d'Ivoire, Myanmar, Uganda and Zimbabwe |
| | Klarkowski et al. [10] | DRC |
| | Kufa et al. [39] | South Africa |
| | Learmonth et al. [40] | Multi-country study (26 countries) |
| | Manak et al. [16] | Nigeria |
| | Maparo et al. [41] | Zimbabwe |
| | Martin et al. [42] | USA |
| | Masina et al. [43] | Malawi |
| | Mayaphi et al. [44] | South Africa |
| | Mehra et al. [45] | India |
| | Mine et al. [46] | Botswana |
| | Nelson et al. [47] | Mozambique |
| | Sacks et al. [48] | UK |
| | Shanks et al. [11] | DRC, Burundi and Ethiopia |
| | Shanks et al. [13] | Ethiopia |
| Shanks et al. [12] | Ethiopia | |
| Simoncini et al. [49] | Niger | |
| Stetler et al. [50] | Honduras | |
| Tchounga et al. [51] | Burkina Faso, Cote d'Ivoire and Mali | |
| Wolpaw et al. [52] | South Africa | |
| Viani et al. [53] | USA and Mexico | |
| Young et al. [54] | Mozambique | |
| Focus on misdiagnosis of HIV-negative serostatus | Bassett et al. [55] | South Africa |
| | Kahemele et al. [56] | Tanzania |
| | Matambo et al. [57] | Zimbabwe |
| Olaru et al. [58] | Zimbabwe | |
| General quality issues from sites conducting HIV testing services | Adebayo et al. [59] | Nigeria |
| | Benzaken et al. [60] | Brazil |
| | Bile et al. [61] | Botswana |
| Cham et al. [62] | 30 countries in Africa | |

Table 1. (Continued)

| Category | Study | Location |
|---|------------------------|------------------------|
| General quality issues from sites conducting HIV testing services (Continued) | Iwe et al. [63] | Nigeria |
| | Kalou et al. [64] | Uganda and Tanzania |
| | Kitheka et al. [65] | Kenya |
| | Kyaw et al. [8] | Myanmar |
| | Louis et al. [66] | Haiti |
| | Lali et al. [67] | Uganda |
| | Manyazewal et al. [68] | Ethiopia |
| | Mashauri et al. [69] | Tanzania |
| | Mwangala et al. [70] | Zambia |
| | Ntim et al. [71] | Ghana |
| | Ocheng et al. [72] | Tanzania |
| | Plate et al. [5] | 11 countries in Africa |
| | SEAD [7] | South Africa |
| | Sushi et al. [73] | India |
| Tegbaru et al. [74] | Ethiopia | |

DRC: Democratic Republic of Congo.

Table 2. Reported HIV testing errors and factors potentially related to misdiagnosis

| Category | No. of Studies |
|---|----------------|
| Incorrect/suboptimal testing strategy or algorithm (e.g. testing strategies not aligned to the World Health Organization recommendations, such as a tiebreaker or parallel testing strategies, use of a single RDT to make an HIV-positive diagnosis) | 37 |
| User error (e.g. errors performing RDT or interpreting results, misapplication of buffer, inaccurate reading time and other human errors) | 25 |
| Poor or inadequate management and supervision (e.g. work load stress, staff shortages, lack of training, poor adherence to testing strategy or testing algorithm, substandard operating procedures, testing in window period) | 21 |
| Other factors (e.g. acute infection, cross-reactivity, known HIV status/on ART) | 18 |
| Clerical/technical errors (e.g. mislabelling, poor record-keeping and clerical mistakes) | 16 |
| Weak reactive test results (e.g. faint or ghost lines appearing on test strip) | 14 |

RDT: rapid diagnostic test; ART: antiretroviral therapy.

Table includes 63 reporting studies. One study (Bile et al. 2017) did not report a specific factor or error related to misdiagnosis. Some studies reported multiple factors related to poor quality testing and factors that could be related to potential misdiagnosis.

window period without referring clients for retesting [32,45], HIV testing performed by undertrained or ineligible staff [7,31,59,64,72], low levels of retesting to verify diagnosis before ART initiation [43], poor participation in external quality assessment (EQA) schemes [62], poor site-level supervision [65] and poor adherence to standard operating procedures [7,35,52,59,67,69].

Sixteen studies reported clerical errors [8,11,26,28,29,31,34,35,45,50,63–65,67,73,75]. Errors included poor record-keeping [35], data reporting problems, labelling and transcription mistakes [73] and specimen mix-ups. Poor record-keeping, according to one study, resulted in nearly 30% of errors leading to incorrect status [67]. Clerical

errors were not always clearly defined and may not have always led to misdiagnosis [28].

Fourteen studies reported challenges related to weak reactive test results, particularly difficulty with interpretation [8,10,24,27,30,32,36,38,40,42,44,48,62,74]. A study, which assessed the proficiency of laboratory technicians, found that specimens with very weak levels of HIV-1/2 antibodies were less accurately reported [40]. In Uganda, two studies found that the majority of false reactive results came from weak reactive RDTs [32,36]. A study from the UK that assessed the visual depiction of false reactive and true positive readings reported that most false reactive specimens had a fainter test line than true positive specimens

[48]. Two studies reported incorrect reading of weak reactive bands contributed to the misdiagnosis [10,11].

Eighteen studies reported on several other testing errors and factors potentially related to misdiagnosis. Nine of these studies reported cross-reactivity either between RDTs within an algorithm or with population and individual characteristics [10,22,24,25,27,32,38,56,60]. One study suggested that cross-reactivity between assays used within an algorithm resulted in false positive statuses [27]. Another hypothesized that cross-reactivity may present as weak reactive lines and thereby cause misdiagnosis [32]. Six studies [10,11,25,38,56,60] reported potential issues with RDTs interacting with characteristics of individuals undergoing testing [10,11,38], including having low levels of HIV-1/2 antibodies due to late stage HIV infection [56,60] and exposure of assays to adverse environmental conditions during storage and use [25,38].

Additionally, six studies reported that a proportion of false negative diagnoses were among people with a known HIV status who were on ART [14,16,39,44,47,58]; one of these studies was among children on ART retested using an oral fluid-based HIV RDT [58]. And three studies reported false negative results were due to patients testing in the window period [45] or with acute or early infection [16,44]. For instance, in South Africa, 0.04% (95% CI: 0.0–0.001) and 0.3% (95% CI: 0.1–0.4) of clients with a false negative diagnosis using serology tests were later found to have acute or early HIV infection after retesting with nucleic acid testing technologies [44].

False positive diagnostic errors and misdiagnosis rates

Thirty studies reported on false positive diagnostic errors (43 reports; $n = 16,777$ total positive diagnoses). In general, error rates were small (median: 3.1%; IQR: 0.4%–5.2%) with the exception of a few studies where a tiebreaker test was used to resolve discrepant results [10–14,23,32,33,37,39,41,44,46,47,75] (Figure 2). Of these, six studies (eight reports) exclusively among people with HIV enrolled in care or ART reported that between 0.1% (95% CI: 0–0.3) and 6.6% (95% CI: 4.5–9.6) of people were misdiagnosed (median: 1.6%, IQR: 0.3–4.7%) [10–13,33,37,39,41,47] (Table 3).

In studies reporting false positive diagnoses, nearly all reported the use of a suboptimal testing strategy [11–13,33,39,49,75]. Sixteen studies reported the use of a tiebreaker testing strategy were related to false positive HIV diagnoses [5,12,13,16,24,25,27,29,30,32,34,36,37,68]. In one of these studies, 95% (123/129) of false positive statuses resulted specifically from using a tiebreaker test [32]. Additionally, one study which reported misdiagnosis rates in Burundi, Democratic Republic of Congo (DRC) and Ethiopia reported some clients may have been provided an HIV-positive diagnosis based on a single HIV RDT [11].

False negative diagnostic errors and misdiagnosis rates

Twenty-eight studies reported on false negative diagnoses (40 reports, total negatives = 55,626) (median: 0.4%, IQR 0–3.9%) (Figure 3) [10,12,13,37,39,47,55–57]. The studies reporting the highest proportions, for example, Olaru et al., which was designed to assess how ART impacts test

performance [58], of false negative diagnoses were exclusively among people with HIV on ART who were retested using an HIV RDT-based algorithm.

Nearly all studies reporting false negative diagnoses also reported using a suboptimal testing strategy. Four studies in South Africa reporting false negative diagnoses reported that HIV testing was conducted with an algorithm using a first-line test with high specificity and poorer sensitivity [14,33,39,55]. According to one of these studies [14], between 2014 and 2016, the testing algorithm changed four times in an effort to address the high proportion of false negative diagnoses resulting from these algorithms.

Clerical and user errors [57], early/acute infection [16,44,45], presentation late in disease stage [56] and individuals with known HIV status on ART who sought retesting, or were retested using oral fluid-based RDTs [58], were also reported as factors contributing to false negative diagnostic errors [14,16,39,44,47]. In Zimbabwe, all the reported false negative diagnoses were among children on ART who were retested with an oral fluid-based HIV RDT [58]. In South Africa and Zambia, individuals on ART comprised 44% (26/59) and 14% (5/38) of false negative diagnoses, respectively [14]. In Mozambique, 88% (21/24) of all true HIV-positive clients with a false negative test result were confirmed to know their HIV status and 62% (13/21) were reportedly on ART [47]; reasons for retesting in study reportedly included users misunderstanding the question or hoping to receive health services and emotional or mental health issues.

Discussion

This review identified and described a number of diagnostic errors and poor HIV testing practices that may lead to misdiagnosis. Data on the magnitude of misdiagnosis was identified but limited, and no study could determine or quantify the exact cause(s) of misdiagnosis. Although no studies could determine and quantify the exact cause(s) of misdiagnosis, several identified the following factors to have strongly contributed: (1) suboptimal testing strategies, (2) poor management of supplies, (3) user errors including difficulty interpreting weak reactive lines and (4) retesting among people with known HIV status on ART.

No assay is perfect. False reactive and false non-reactive results are inevitable when using a single RDT and should be anticipated. However, the risk of misdiagnosis should be very low when a validated testing algorithm for high ($\geq 5\%$) or low ($< 5\%$) prevalence settings is used [76]. In this review, we identified that many studies reporting diagnostic errors - both false positive and false negative - utilized suboptimal testing strategies which were not aligned to international guidance. Studies reviewed clearly showed the use of a tiebreaker strategy to rule-in HIV infection increases the likelihood of false positive statuses and possible misdiagnosis. This is concerning because a third of national testing strategies reviewed in 2015 recommended using a tiebreaker testing strategy [9].

In addition to adopting a proven testing strategy, national or regional validation is critical to determine which RDTs, and in which order, perform the best as a complete algorithm. As previously reported [38,77–83], tests and

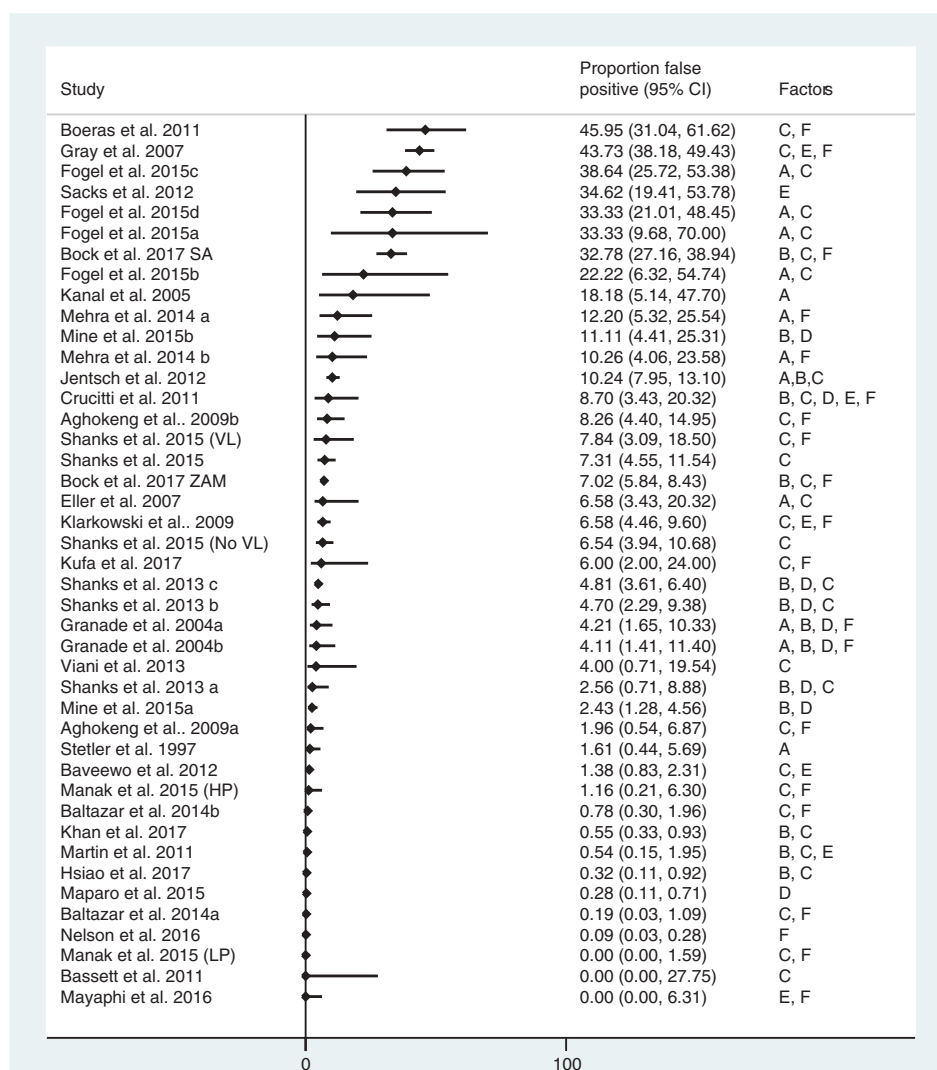


Figure 2. Rates of false positive diagnostic error rates diagnosis (n = 30 studies, 43 reports).

LP: low prevalence; HP: high prevalence; ZAM: Zambia; VL: visceral Leishmaniasis; Data reported include reports of misdiagnosis of HIV-positive statuses. False positive diagnoses were reported in 30 studies (43 reports), total positive diagnoses n = 16,777. Kufa et al. 2017 reported proportion misdiagnosed by did not report full sample size. In studies where all participants were known to be HIV positive and/or on ART at the beginning of the study, the full study population was used as the denominator.

algorithm performance vary across settings, often due to cross-reactivity caused by HIV subtypes, co-infections, comorbidities and possible environmental or population characteristics. Without validating a testing algorithm at a country or regional level, it would not be possible to fully understand the causes of poor performance. Furthermore, to ensure correct diagnoses, it is important to retest people diagnosed HIV positive before they enrol in care and ART. This is a cost-effective approach [84] which is increasingly critical as more people with HIV are being offered immediate treatment.

To ensure correct results, all staff providing HIV testing must be trained, certified and provided ongoing support and supervision. In several studies, this was not the case,

and untrained and uncertified providers were performing HIV testing [7,72]. Training, including pre-service, in-service and periodic refresher training, is important to maintain and improve the quality of services. Participation in EQA schemes is another way to monitor performance and improve testing services. Several studies also reported user and clerical errors resulted from inadequate support, demanding workloads, burnout and high levels of stress [11,62,64,66]. Adequate support and supervision are critical to reduce stock outs which may contribute to the use of damaged or expired test kits, incorrect test kits and buffer. Sites should routinely assess and manage their supplies and human resource planning to prevent or reduce these circumstances.

Table 3. Rates of false positive diagnosis rates among people diagnosed with HIV and/or enrolled in care or antiretroviral therapy (ART)

| Study/author | Sample size | Total no. of retested | No. of false positives | Percentage of false positive diagnoses |
|------------------------|-------------|-----------------------|------------------------|--|
| Klarkowski et al. 2009 | 365 | 229 | 24 | 6.6 |
| Shanks et al. 2013c | 914 | 54 | 44 | 4.8 |
| Shanks et al. 2013b | 149 | 149 | 7 | 4.7 |
| Shanks et al. 2013a | 78 | 78 | 2 | 2.6 |
| Khan et al. 2017 | 2533 | 88 | 14 | 0.55 |
| Hsiao et al. 2017 | 952 | 37 | 3 | 0.3 |
| Maparo et al. 2015 | 1447 | 1447 | 4 | 0.28 |
| Nelson et al. 2016 | 3160 | 3146 | 3 | 0.1 |

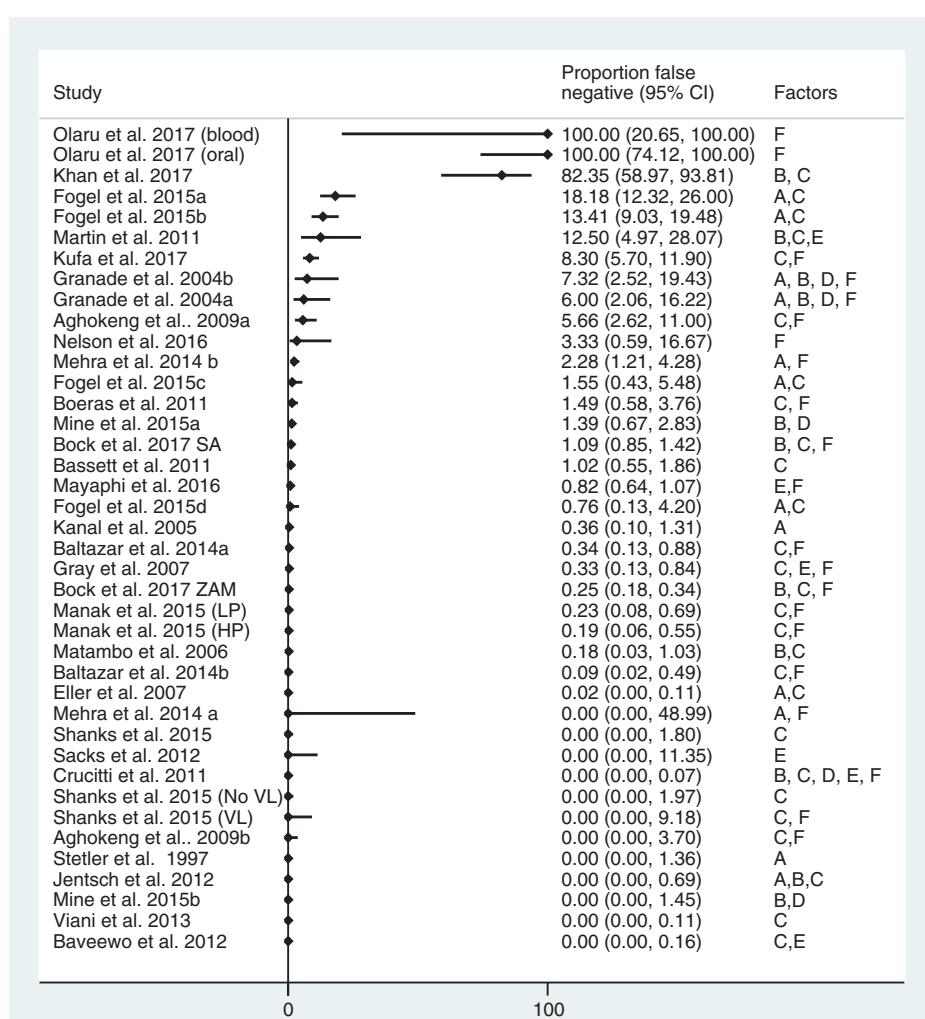


Figure 3. False negative diagnostic error rates ($n = 28$ studies, 40 reports).

LP: low prevalence; HP: high prevalence; SA: South Africa, discrepant results; ZAM: Zambia, discrepant results; VL: visceral Leishmaniasis; Data reported includes reports of misdiagnosis of HIV-negative statuses. Misdiagnoses of HIV-negative statuses were reported 28 studies (40 reports), total negative = 55, 626. Kufa et al. 2017 reported proportion misdiagnosed but did not report full sample size information. Note Olaru et al. was exclusively among people with HIV on ART, accounting for the high rate of false negative diagnoses.

User error interpreting weak reactive lines was a common challenge which contributed to false positive results. To address this, specialized training for health workers and site-level standard operating procedures including the use of a “second-reader” to validate the correct interpretation of test results may be needed, as well as work with manufacturers to improve RDTs and instructions on how to interpret faint lines and weak control lines. Several studies hypothesized that weak reactive lines may be caused by other user errors, for example, misapplication of buffer and reading test results too early and cross-reactivity. Further investigation into the cause of weak reactive and other faint lines, and how they can be prevented, is needed.

False negative test results among people with HIV and on ART were observed and contributed to a substantial proportion of misdiagnoses [14,16,39,44,47]. While it is unclear why people on ART would seek retesting, some reports suggest it may be due to wanting to “check” or “confirm” one’s HIV status and religious beliefs about being “cured” [85], as well as misunderstandings and emotional or mental health issues [47]. It is important for programmes and users to be aware of the potential risk of false negative results, as the presence of ART can lead to confusing test results and could result in individuals unnecessarily stopping treatment which could have dire individual and public health implications. As “treat all” policies are rolled out, it will be increasingly critical for programmes to address this issue and ensure clients and health workers are aware that testing individuals on ART is not recommended [76].

Strengths and limitations

This analysis is the first to bring together a diverse set of studies with the aim of identifying and describing suboptimal HIV testing practices and misdiagnosis. The results indicate the problem of misdiagnosis deserves attention. However, there are several limitations to this review.

As with all literature reviews, publication bias may be an issue and for this topic is inevitable and information on misdiagnosis is often unreported. This review was also limited to reports in English and may have missed reports in other languages. The majority of reports are from Africa and may not be representative of other geographies. Because the review was designed to identify reports of misdiagnosis, it is possible studies reporting errors and quality of HIV testing may have been missed.

Due to both the paucity and heterogeneity of data, it was not possible to conduct more quantitative analyses. Studies included were generally not designed to determine the exact cause or causes of misdiagnoses, a weakness cited across research on diagnostic errors [86].

This review focused on human errors and quality system failures. While we did identify some reports of cross-reactivity [10,22,24,25,27,32,38,56,60], reports did not provide conclusive information on what exactly caused cross-reactivity. Possible biological factors due to antibodies from inter-current infections, adverse environmental exposure to assay components, HIV subtype or shared false cross-

reactivity in RDTs within an algorithm may be issues requiring further investigation.

Acute and early infection did not appear to be a significant cause of false negative diagnoses; however, few studies identified reported on acute infection. Retesting among HIV-positive individuals taking ART did emerge as a key factor contributing to a substantial proportion of false negative diagnostic errors and misdiagnoses. Further research is needed to understand how ART, as well as the use of antiretroviral drugs for prevention, for example, pre-exposure prophylaxis, may impact the performance of HIV RDTs, as well as how frequently people previously diagnosed with HIV and on ART retest.

Conclusions

Our review has identified a number of factors and practices that may contribute to diagnostic error and HIV misdiagnosis. Although no study could fully determine and quantify the exact cause(s) of misdiagnosis, our review elucidated four key factors: (1) suboptimal testing strategies, primarily the use of a tiebreaker testing strategy to rule in HIV infection, (2) user errors including interpretation of weak reactive lines, (3) inadequate management and supervision of testers and (4) retesting among people with HIV on ART. Most, if not all, are avoidable with appropriate guidelines, training and supervision. The consequences of misdiagnoses are serious at an individual and public health level. With the momentum to scale-up HIV diagnosis and linkage to ART, a parallel push to improve the quality of HIV testing services and prevent misdiagnosis is essential.

Authors’ affiliations

¹Department of HIV, World Health Organization, Geneva, Switzerland; ²Department of Clinical Research, London School of Hygiene and Tropical Medicine, London, UK; ³Department of Psychiatry and Behavioral Sciences, Medical University of South Carolina, Charleston, SC, USA; ⁴Department of Essential Medicines and Health Products, World Health Organization, Geneva, Switzerland; ⁵Department of Epidemiology and Population Health, American University of Beirut, Beirut, Lebanon; ⁶Department of International Health, Johns Hopkins University, Bloomberg School of Public Health, Baltimore, MD, USA; ⁷US Agency for International Development, Washington, DC, USA

Competing interests

The authors declare no competing interests. The authors alone are responsible for the views expressed in this article and they do not necessarily represent the views, decisions or policies of the institutions with which they are affiliated.

Authors’ contributions

RB, CMO, CJ and AS conceived of and provided overall guidance to the study. VF and ST conducted the primary literature review and drafted the initial report. VF and CJ conducted the update of the literature review and data extraction. CJ primarily drafted the manuscript, performed quality assessment and data analyses and undertook supplementary literature reviews. NF and CJ conducted the analyses. All authors have contributed to the conceptualization of the study, contributed to the development of drafts and read and approved the final version.

Funding

This publication was supported in part by a grant from the US National Institutes of Health (NIH; 5 R01 HD053268), Carla Makhlof Obermeyer, PI,

and the U.S. Agency for International Development (USAID), with support from the U.S. President's Emergency Plan for AIDS Relief. The content of this supplement is solely the responsibility of the authors and does not necessarily represent the official views of the USA Government or the World Health Organization.

Disclaimer

The funders of the study had no role in study design, data collection, data analysis, data interpretation or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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Research article

Understanding low sensitivity of community-based HIV rapid testing: experiences from the HPTN 071 (PopART) trial in Zambia and South Africa

Peter Bock^{1§}, Comfort Phiri², Estelle Piwovar-Manning³, Barry Kosloff^{2,4}, Nomtha Mandla¹, Alicia Young⁵, Anelet James¹, Ab Schaap^{2,6}, Michelle Scheepers¹, Deborah Donnell⁵, Sam Griffith⁷, Wafaa El-Sadr⁸, Kwame Shanaube², Nulda Beyers¹, Richard Hayes⁶, Sarah Fidler⁹, Helen Ayles^{2,4} and on behalf of the HPTN 071 (PopART) Study Team

[§]Corresponding author: Peter Bock, Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Stellenbosch University, Francie van Zijl Avenue, Clinical Building, K Floor, Room 0065, Tygerberg Campus, Western Cape 7505, South Africa. (peterb@sun.ac.za)

Abstract

Introduction: Population-wide HIV testing services (HTS) must be delivered in order to achieve universal antiretroviral treatment (ART) coverage. To accurately deliver HTS at such scale, non-facility-based HIV point-of-care testing (HIV-POCT) is necessary but requires rigorous quality assurance (QA). This study assessed the performance of community-wide HTS in Zambia and South Africa (SA) as part of the HPTN 071 (PopART) study and explores the impact of quality improvement interventions on HTS performance.

Methods: Between 2014 and 2016, HIV-POCT was undertaken within households both as part of the randomly selected HPTN 071 research cohort (Population Cohort [PC]) and as part of the intervention provided by community HIV-care providers. HIV-POCT followed national algorithms in both countries. Consenting PC participants provided a venous blood sample in addition to being offered HIV-POCT. We compared results obtained in the PC using a laboratory-based gold standard (GS) testing algorithm and HIV-POCT. Comprehensive QA mechanisms were put in place to support the community-wide testing. Participants who were identified as having a false negative or false positive HIV rapid test were revisited and offered retesting.

Results: We initially observed poor sensitivity (45–54%, 95% confidence interval [CI] 31–69) of HIV-POCT in the PC in SA compared to sensitivity in Zambia for the same time period of 95.8% (95% CI 93–98). In both countries, specificity of HIV-POCT was >98%. With enhanced QA interventions and adoption of the same HIV-POCT algorithm, sensitivity in SA improved to a similar level as in Zambia.

Conclusions: This is one of the first reports of HIV-POCT performance during wide-scale delivery of HTS compared to a GS laboratory algorithm. HIV-POCT in a real-world setting had a lower sensitivity than anticipated. Appropriate choice of HIV-POCT algorithms, intensive training and supervision, and robust QA mechanisms are necessary to optimize HIV-POCT test performance when testing is delivered at a community level. HIV-POCT in clients who did not disclose that they were on ART may have contributed to false negative HIV-POCT results and should be the topic of future research.

KEYWORDS: HIV rapid test; community; household; sensitivity; quality control; HPTN 071 (PopART)

To access the supplementary material to this article please see [Supplementary Files](#) under Article Tools online.

Received 22 December 2016; Accepted 25 June 2017; Published 29 August 2017

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Introduction

Globally, 37 million people are estimated to be living with HIV [1]. In 2014, UNAIDS announced a global target of 90% of HIV-positive individuals knowing their HIV status in order to deliver universal access to antiretroviral treatment (ART) for all people living with HIV (PLWH) [2]. However, there remains a considerable HIV testing gap, with only 54% of PLWH aware of their HIV status in 2014 [3]. Reaching the UNAIDS 90-90-90 targets will require a massive scale-up of

HIV testing and will necessitate innovative strategies to achieve this goal.

Whilst HIV testing services (HTS) are usually provided at healthcare facilities, multiple barriers prevent wide-scale access and acceptance of testing through this approach [4]. To improve knowledge of HIV status, non-facility-based HIV testing approaches have been explored [5,6] and many are now integrated into community testing programmes [5,7–9]. Previous studies have shown high levels

of competency in HIV testing amongst counsellors in household settings [10,11], and high levels of acceptance for community-based HIV testing are reported [5]. However, the quality assurance (QA) of this mode of HIV testing may be more challenging. The sensitivity and specificity of HIV point-of care testing (HIV-POCT) may be affected by user training and competency, testing environments, the algorithm used, test kit handling and storage as well as test kit performance [12–14]. Sensitivity and specificity of commonly used HIV-POCT in laboratory conditions are high (consistently 97–99%) [5,15,16]. However, there are limited and varied data on the performance of HIV-POCT in field settings, and comparison to a laboratory-based gold standard (GS) is uncommon [15,16]. The World Health Organization (WHO) pre-qualifies certain HIV testing strategies [7], but countries may utilize algorithms based on price and availability of test kits.

HIV-POCT QA guidelines vary across settings. The WHO emphasizes the importance of QA supported by well-structured quality management services and has recently updated its guidance for establishing HIV testing QA. The WHO recommends using a combination of quality control (QC) of HIV test kits and monitoring of proficiency of the staff conducting tests using both internally and externally generated plasma panels [7]. Effective implementation of these guidelines is resource-intensive and requires basic equipment and laboratory infrastructure that may be difficult to access in many high-burden settings [7].

HPTN 071 (PopART) is a community-randomized trial investigating the impact of a combination HIV prevention package on HIV incidence. The design of the study has been reported previously [17]. A key component of the combination prevention package is community-wide HIV testing offered by a novel cadre of community HIV-care providers (CHiPs) within the households of consenting individuals using HIV-POCT. CHiPs workers are “lay counsellors” who have a minimum of grade 11 or 12 high school education prior to employment and received basic accredited HIV counselling and testing training prior to conducting HIV-POCT in the field. In parallel with the CHiPs HIV testing, a randomly selected research Population Cohort (PC) of participants consented to provide an annual blood sample to determine HIV status in study laboratories for the study’s primary endpoint; many of these individuals also accept optional HIV-POCT delivered by research nurses in their households. This cohort provides an opportunity to assess performance of community-wide HIV-POCT compared to a laboratory-based GS. This manuscript describes the performance of community-wide HIV-POCT in Zambia and South Africa (SA) as part of the HPTN 071 (PopART) study.

Methods

Within each of the 21 communities in Zambia and SA included in the HPTN 071 (PopART) study, a random sample of approximately 2000 participants aged between 18 and 44 years were selected to join the PC. Consenting participants were visited in their households and asked to provide

a venous sample of blood for laboratory-based HIV testing (blinded for study arm) to inform the study primary endpoint (HIV incidence). Results of this laboratory HIV testing were not routinely returned to study participants. All participants were encouraged to undergo HIV-POCT using the current nationally approved test algorithm. The results of this testing were given directly to the participant. Not all PC participants chose to have a HIV-POCT; some may already have been tested by the CHiPs or have previously known their status. For this paper, data from the baseline survey of the PC (PC0) and the 12-month follow-up survey (PC12) were analysed.

HIV-POCT testing algorithms

In both Zambia and SA, HIV-POCT was undertaken by both trained CHiPs (lay counsellors) for the community combination prevention intervention and research nurses for the PC. In both cases, two HIV-POCT tests performed in series were used, in line with national and local guidelines. In Zambia, the Alere Determine™ HIV-1/2 test (Alere inc., CA, USA) was used for screening and the Uni-Gold HIV test (Trinity Biotech, Bray, Co.Wicklow, Ireland) was used for confirmation throughout the study period.

In SA testing followed the national algorithm which varied during the study period. From January to June 2014, the First Response™ HIV 1-2-0 Card Test (Real Relief India Private Limited, Tamil Nadu, India) was used for screening and the Alere Determine™ HIV-1/2 for confirmation; from July to December 2014, SD Bioline HIV-1/2 3.0 (Alere, CA, USA) for screening and Alere Determine™ HIV-1/2 test was used for confirmation; from January to June 2015, the ADVANCED QUALITY™ Rapid Anti-HIV (1&2) Test (InTec Products Inc., Haicang, Xiamen, China) was used for screening and the Abon HIV 1/2/O Tri-line test (Alere Inc., CA, USA) was used for confirmation. These changes in tests kits matched those of the SA Department of Health (SADOH) which provided the study with test kits during that period.

Following the analysis of the performance of these HIV-POCT algorithms, the study team chose to provide kits for SA HIV-POCT from July 2015 onwards such that Alere Determine™ HIV-1/2 test was used for screening and the Uni-Gold™ Recombigen® HIV-1/2 test was used for confirmation, to be consistent with the algorithm used in Zambia.

HIV-POCT quality management

A system of quality management for the HIV-POCT was developed which included both QC for the test kits and QA of the testing procedure (QA/QC). This system used nationally available guidelines, but was expanded by the study team to include internal quality control (IQC) panel testing of test kits, temperature monitoring of test kits and proficiency testing of all staff conducting HIV testing. In Zambia, additional procedures were established earlier than in SA, as initially the SA test kits were provided by the DOH and QC systems used by DOH were assumed to be adequate. The timing of the implementation of additional procedures by the study team is shown in Table 1. Details of the additional procedures are as follow:

Table 1. Performance, test kits used and quality measures in Zambia (Z) and South Africa (SA)

| | Q1 2014 | Q2 2014 | Q3 2014 | Q4 2014 | Q1 2015 | Q2 2015 | Q3 2015 | Q4 2015 | Q1 2016 | Q2 2016 |
|---|-----------------|-----------------|-----------------|-----------------|-----------------|----------------|-----------------|-----------------|-----------------|-----------------|
| Zambia | | | | | | | | | | |
| N (total test) | 1317 | 2038 | 2346 | 2318 | 2103 | 0 ^a | 822 | 2002 | 2194 | 1140 |
| Correctly identified HIV-positive (HIV-POCT+/GS +) | 229/238 | 231/248 | 197/221 | 213/235 | 146/157 | | 48/51 | 125/130 | 124/130 | 70/74 |
| Correctly identified HIV-negative (HIV-POCT-/GS-) | 1077/1079 | 1788/1790 | 2121/2125 | 2081/2083 | 1944/1946 | | 767/771 | 1871/1872 | 2063/2064 | 1065/1066 |
| Sensitivity % (95% CI) | 96.2 (93–98) | 93.1 (89–96) | 89.1 (84–93) | 90.6 (86–94) | 93.0 (88–96) | | 94.1 (84–99) | 89.9 (84–94) | 95.4 (90–98) | 94.6 (87–98) |
| Specificity % (95% CI) | 99.8 (99.3–100) | 99.9 (99.6–100) | 99.8 (99.5–100) | 99.9 (99.7–100) | 99.9 (99.6–100) | | 99.5 (98.6–100) | 99.9 (99.6–100) | 100 (99.7–100) | 99.9 (99.5–100) |
| Zambia first-line POCT | Determine | Determine | Determine | Determine | Determine | | Determine | Determine | Determine | Determine |
| Zambia second-line POCT | Uni-Gold | Uni-Gold | Uni-Gold | Uni-Gold | Uni-Gold | | Uni-Gold | Uni-Gold | Uni-Gold | Uni-Gold |
| IQC test strips/devices (pass/tested) | 67/67 | 95/95 | 615/615 | 752/752 | 423/423 | 1164/1164 | 2840/2840 | 2874/2874 | 3543/3543 | 2528/2528 |
| Panel proficiency testing (pass/total) | 96/99 | 96/99 | 79/82 | 143/151 | 98/100 | 108/110 | 141/144 | 120/124 | 55/55 | 102/106 |
| South Africa | | | | | | | | | | |
| N (total test) | 429 | 672 | 395 | 90 | 453 | 0 ^a | 1029 | 911 | 973 | 436 |
| Correctly identified HIV-positive (HIV-POCT +/GS +) | 13/24 | 21/43 | 9/20 | 3/3 | 16/23 | | 38/52 | 33/42 | 16/21 | 13/13 |
| Correctly identified HIV-negative (HIV-POCT-/GS-) | 405/405 | 629/629 | 375/375 | 87/87 | 430/430 | | 977/977 | 868/869 | 952/952 | 423/423 |
| Sensitivity % (95% CI) | 54.2 (33–74) | 48.8 (33–65) | 45.0 (23–68) | | 69.6 (47–87) | | 73.1 (59–84) | 78.6 (63–90) | 76.2 (53–92) | 100 (75–100) |
| Specificity % (95%CI) | 100 (99–100) | 100 (99–100) | 100 (99–100) | 100 (96–100) | 100 (99–100) | | 100 (99.6, 100) | 99.9 (99–100) | 100 (99.6, 100) | 100 (99–100) |
| SA first-line POCT | First response | First response | SD Bioline | SD Bioline | Advance quality | | Advance quality | Determine | Determine | Determine |
| SA second-line POCT | Determine | Determine | Determine | Determine | Abon | | Abon | Uni-Gold | Uni-Gold | Uni-Gold |
| IQC test strips/devices (pass/tested) | | | | | 1482/1482 | 556/556 | 718/718 | 2297/2297 | 2131/2131 | 3090/3090 |
| Panel proficiency testing (pass/total) | | | | | 4/4 | 119/122 | 129/130 | 43/43 | 32/34 | 0 |

HIV-POCT+: final result of HIV-POCT algorithm is positive; HIV-POCT-: final result of HIV-POCT algorithm is negative; GS+: final result of laboratory algorithm is positive; GS-: final result of laboratory algorithm is negative.

HIV-POCT: HIV point-of-care testing; IQC: internal quality control; GS: gold standard; QA: quality assurance; PC: Population Cohort.

^aNo PC activity this quarter but QA continued.

^bSensitivity not calculated due to small number of positive results.

IQC panel testing of test kits was performed (i) when new test kits were delivered to study head office, (ii) after transport of test kits to site offices within the communities and (iii) monthly for test kits that had been stored at site offices and transported in the field. Due to the large number of test kits used, panels used for IQC testing were generated by each in-country study laboratory. In Zambia, IQC activities described in this paper were initiated at the beginning of the study whilst in SA QC of test kits was conducted by the SADOH initially but was undertaken by the study team from Q1 2015 onwards.

Temperature monitoring during test kit storage was conducted in each country at the in-country study head office, at field offices and in cooler boxes that were used to transport HIV-POCT kits in the field. In instances where out-of-range temperatures were reported ($>27^{\circ}\text{C}$ for three consecutive days), IQC was performed for the affected test kits as described above.

User proficiency to perform the HIV-POCT kit procedures according to the manufacturers' specifications was assessed among all PC research staff and among CHiPs. In both countries, PC research nurses and CHiPs completed regular internal and external proficiency testing (EQA).

A checklist was developed to be used for observation of all staff performing HIV-POCT. This checklist covered all aspects of home-based testing, including: preparing the testing environment, obtaining a finger stick sample, carrying out testing and interpreting results (see Appendix). In addition, in both countries, internal proficiency panel testing was done with blinded plasma panels of HIV-positive and HIV-negative samples at least once per year for all testers. EQA with samples provided by the National Health Laboratory Service (NHLS) in SA and the National Virology Reference Laboratory (NVRL) in Zambia was also conducted on an annual basis from 2015 when these panels were made available.

If an individual staff member failed internal- or external proficiency testing, the individual underwent re-training and repeat proficiency testing before being allowed to resume HIV testing.

Laboratory-based HIV testing

In this large clinical trial, special algorithms were developed for laboratory-based HIV testing in the PC. In addition to HIV-POCT described above which was part of the study intervention, venous blood was collected from each PC study participant for laboratory-based testing to provide data for the primary study endpoint of HIV incidence. This testing was done in two stages. In the first step, a single HIV screening assay (Abbott Architect Combo) was performed in-country. The results of that test dictated the algorithm that was used at the HPTN Laboratory Center (HPTN-LC, Johns Hopkins Univ. School of Medicine, Baltimore, MD, USA) for QA and HIV confirmation. For 10% of the samples where the in-country test was non-reactive, testing was repeated at the HPTN-LC with the same 4th generation test (the Abbott Architect Combo). If the results of the two tests were discrepant, samples were tested with the 4th generation Bio-Rad HIV 1/2 Combo (Bio-Rad Combo test) and the Bio-Rad Geenius discriminatory assay. For all

samples that had a reactive in-country test, testing was performed at the HPTN-LC with a different 4th generation test (the Bio-Rad 4th generation assay). If the in-country and HPTN-LC test results were discrepant, samples were tested at the HPTN-LC with Abbott Architect assay, the Bio-Rad Geenius discriminatory assay and HIV viral load testing. The final HIV status determined at the HPTN-LC is defined in this paper as the GS. Results of HIV tests performed in the in-country laboratories and at the HPTN-LC were not reported to study participants, unless discrepancies were identified between HIV-POCT among those who accepted the testing and final laboratory test results.

Management of discrepant results between laboratory test and HIV-POCT

In both countries, PC participants who had discrepant results for the laboratory-based test and HIV-POCT were revisited by the research staff and offered the opportunity for repeat HIV testing using HIV-POCT; this was followed by collection of an additional venous blood sample in cases where the HIV-POCT was still discrepant with the laboratory result. Information was also collected regarding prior knowledge of HIV status, engagement in care if aware of HIV-positive status and ART at the time of initial HIV-POCT.

Data management and statistical analysis

Data for all PC participants were collected electronically using a specially designed database. All participants were identified by a unique barcode. HIV-POCT results were recorded first on a barcoded paper-based results form by the nurse, and this information was entered into the electronic data capture device at the end of each day by the research assistant. All blood samples were labelled using the participant barcode and sent to laboratories for processing within 6 h of blood draw. Aliquots of plasma were stored at -80°C until laboratory testing. All laboratory data were entered into a laboratory data management system. In the case of discrepant results between laboratory test and HIV-POCT, data entry errors were excluded by retrieval of the source document HIV-POCT form and comparison and correction on the electronic data base.

This analysis of performance of HIV-POCT compared to a laboratory reference standard was limited to those PC participants with both an HIV-POCT result and a laboratory HIV test result corresponding to PC visits taking place between January 2014 and June 2016. Estimates of sensitivity and specificity of HIV-POCT over time, with exact binomial 95% confidence intervals (95% CI), were calculated in order to assess the possible effects of test kit choice and improvement in quality management.

Ethical approval

Ethical approval for the HPTN 071 study was obtained from the University of Zambia research ethics committee, Stellenbosch University health research ethics committee and the London School of Hygiene and Tropical Medicine ethics committee.

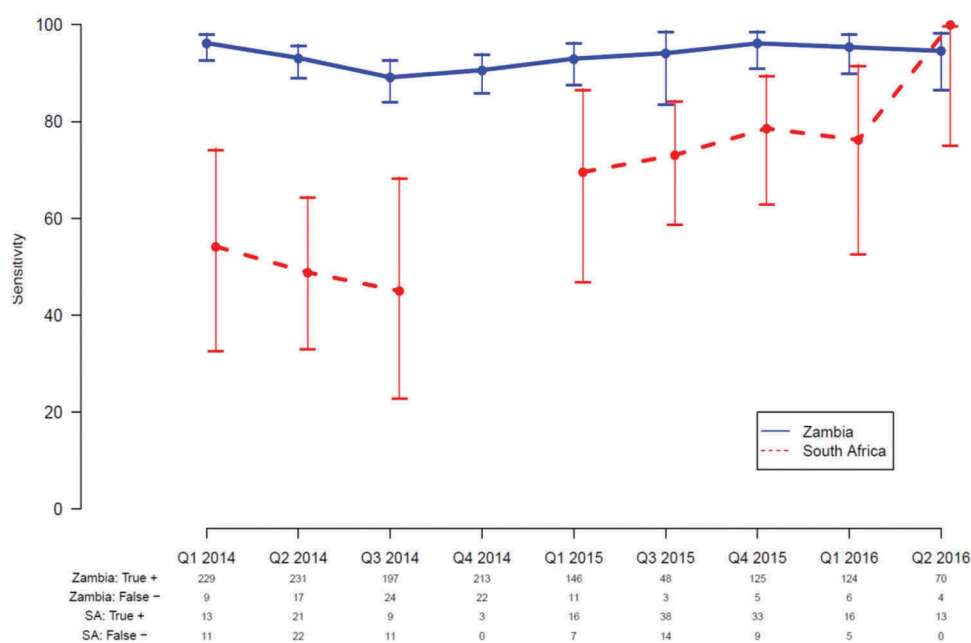


Figure 1. Sensitivity of HIV-POCT in Zambia and South Africa by quarter. HIV-POCT: HIV point-of-care testing.

Results

Study population

Data analysed in this paper include 21,668 paired HIV-POCT and laboratory GS results obtained from 17,680 PC participants at the PC enrolment and/or 12-month follow-up surveys (16,280, 75.1% Zambia, 5388, 24.9% SA).

HIV-POCT performance

Using data from PC participants who had both HIV-POCT and laboratory results available, we examined HIV-POCT performance over time by quarter. **Figure 1** summarizes HIV-POCT sensitivity for each country. **Table 1** shows sensitivity and specificity by country over time alongside the test kit algorithms and other quality management activities.

Data from Zambia for the entire period showed a sensitivity of 89–96%, with the lower limit of the 95% CI remaining above 84% throughout. However, the sensitivity of HIV-POCT in SA was very different, with observed sensitivity as low as 45%.

In SA the test kit algorithm changed first in Q3 2014 in line with SA national guideline change and again in Q1 2015. Neither of these changes in HIV-POCT algorithm appeared to significantly change the performance of the testing process. As a consequence of continuing poor performance in SA, HIV-POCT algorithm was changed in Q4 2015 to be consistent with that used in Zambia (Alere Determine™ HIV-1/2 followed by Uni-Gold™ Recombigen® HIV-1/2). Additional quality management procedures were also employed to monitor HIV-POCT performance, similar to what was being implemented in Zambia. These included re-training of all staff and more frequent staff supervision. Proficiency testing using approved plasma panels was introduced.

Quality assurance

IQC testing was performed on a total of 25,175 test strips/devices overall at central storage and field sites, as well as when temperature monitoring showed deviations from the recommended storage temperatures in storage sites or field cooler boxes. On all occasions, the test strips/devices tested, passed (IQC) (**Table 1**).

Internal proficiency panel testing was conducted annually so that during this period individual testers may have been tested more than once. A total of 971 proficiency panels were used (934 for CHiPs and 37 for PC nurses) in Zambia with an overall pass rate of 96% (**Table 1**). External proficiency panel testing was conducted once during the period of this report and 419/444 testers (94%) passed (20 PC nurses were tested with 100% pass rate). In SA, internal proficiency panel testing started later and a total of 333 proficiency panels being used (271 for CHiPs and 62 for PC nurses) with an overall pass rate of 98%. All individuals failing proficiency panel testing were re-trained and had to pass a further proficiency panel test before being allowed to resume testing. External panel proficiency testing was conducted in the six HPTN 071 intervention sites with one panel per site being tested rather than individual testers. All six sites were tested on four occasions with one site failing on one occasion. This site received additional re-training.

Observation of all steps in the HIV-POCT process using the supervision checklist started in 2015, and observations using this revealed that most errors were made in the finger stick and correct use of the sample collection device (capillary tube or pipette according to test used). Errors were also made in the timing and amount of chase buffer added.

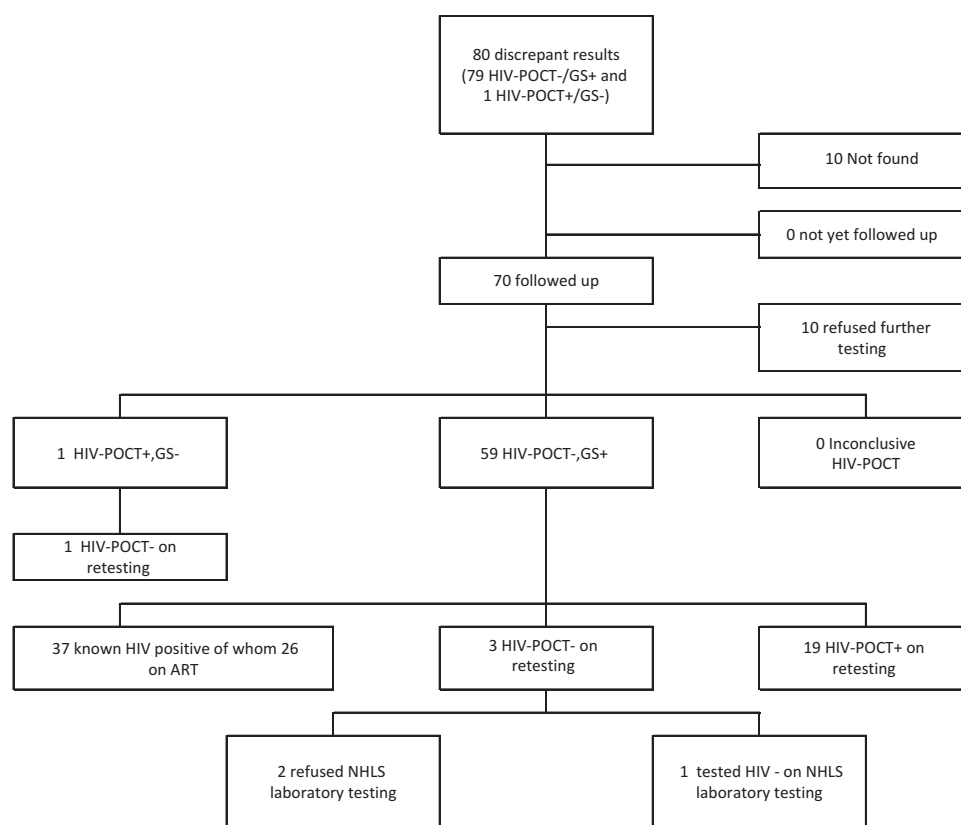


Figure 2a. Flow chart of follow up of participants with discrepant HIV results South Africa.

HIV-POCT: HIV point-of-care testing. HIV-POCT-: original HIV-POCT algorithm negative; HIV-POCT+: original HIV-POCT algorithm positive; inconclusive HIV-POCT-: original HIV-POCT algorithm discordant; GS+: laboratory algorithm (gold standard) HIV positive; GS-: laboratory algorithm negative; GS confirmed: after retesting the HIV-POCT agreed with the laboratory gold standard; HIV-POCT confirmed: after retesting the results of the repeat HIV-POCT algorithm agreed with the original HIV-POCT algorithm.

Follow-up of individuals with discrepant HIV-POCT and laboratory tests

Overall, 199 participants had 200 discrepant HIV results (participants were seen annually so it was possible for them to receive discrepant results in both years). Figure 2 summarizes for each country the follow-up of participants with test results that were discrepant between the HIV-POCT and the laboratory GS. In Zambia 120 and in SA 80 participants were identified with discrepant results. Multiple attempts to revisit all these participants were made by the research teams in both countries, according to a standardized algorithm, during which these participants were offered a repeat HIV-POCT and laboratory test. There were some differences in the procedures for conducting re-test visits between Zambia and SA.

In SA, re-test visits have been attempted for all 80 participants with confirmed discrepant results. PC staff were unable to locate 10 participants, and a further 10 declined a re-test visit, for the remaining 60 participants, 59 appeared to have initial false negative results (HIV-POCT-negative but GS-positive) and 1 an initial false positive result (HIV-POCT-positive but GS-negative). Of the 59 individuals with false negative results, 37 (63%) were found to already know their HIV-positive status and 26 (44%) were

confirmed to be on ART at the time of the false negative POCT. Re-testing was not performed on known HIV-positives; however, they were given adherence counselling and advised to attend the clinic. For the remaining 22 individuals, HIV-POCT was repeated using the algorithm of Alere Determine™ HIV-1/2 and Uni-Gold™ Recombigen® HIV-1/2. Three of these participants again tested HIV-negative on HIV-POCT. Of these, two did not consent to further blood draw for plasma HIV testing and one tested HIV-negative on further in-country laboratory testing. Investigation of this participant was terminated after the participant was lost to follow-up due to relocation out of the study area. Including individuals known to be HIV-positive, a total of 56/59 (95%) were confirmed to have been prior false negative HIV-POCT results. One participant had a false positive HIV rapid test; this participant was re-visited and on re-testing with HIV-POCT tested HIV-negative.

In Zambia, the picture was different. Of the 120 participants with discrepant results, 29 terminated participation at a subsequent PC visit (moved out, not found or refused further participation). Due to delays in laboratory results and receipt of source data from remote sites, the follow-up results of a further 38 participants could not be included. Of the remaining 53 participants followed up, 7 participants

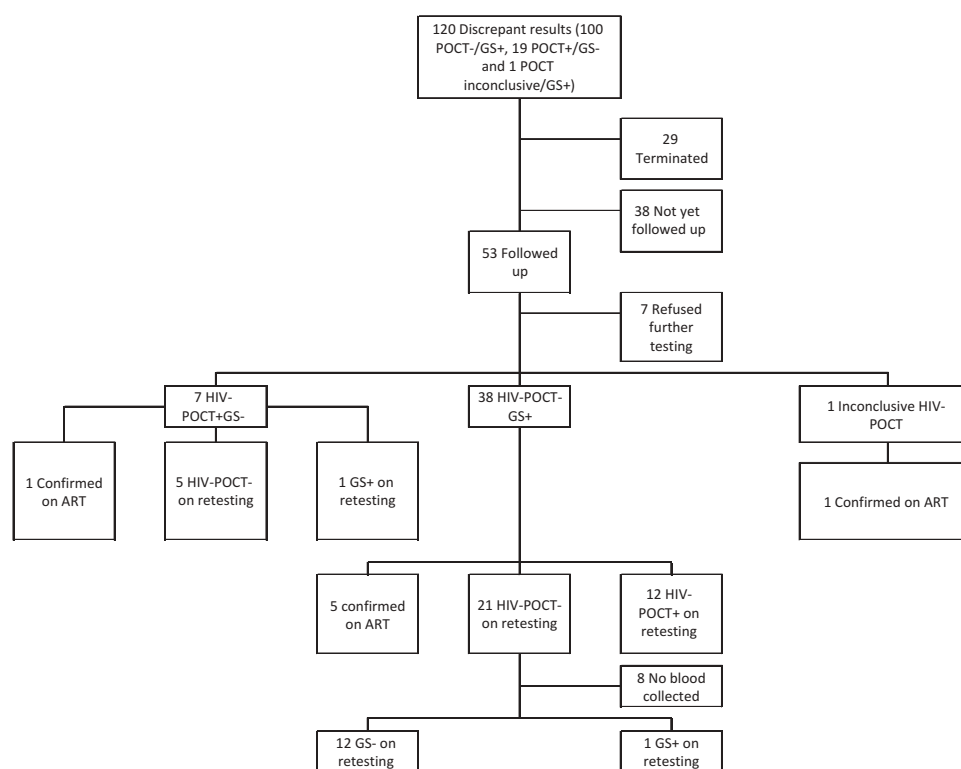


Figure 2b. Flow chart of follow up of participants with discrepant HIV results Zambia.

declined further testing, leaving 46 of whom 38 initially appeared to have false negative HIV-POCT results (HIV-POCT-negative but GS-positive), 7 false positive results (HIV-POCT-positive but GS-negative) and 1 an inconclusive HIV-POCT result (discordant results between the two rapid tests used as the HIV-POCT algorithm, GS-positive). Of the 38 individuals with false negative results, 5 (13%) were already known to be HIV-positive and taking ART. The majority, 21 (55%), had repeat HIV-POCT results consistent with the original negative HIV-POCT, demonstrating some inherent differences between the laboratory and HIV-POCT and some possible laboratory errors. For the remaining 12 (32%), repeat HIV-POCT confirmed the positive laboratory result. For five out of seven apparent false positives, the repeat HIV-POCT was negative, the other two participants were confirmed to be HIV-positive, one participant confirmed that they were on ART and for the other repeat HIV-POCT and laboratory testing confirmed a positive result. Finally, the participant with an inconclusive HIV-POCT stated they were on ART at the follow-up visit.

Discussion

Expanding high-quality community-based HIV-POCT is critical if high burden communities are to achieve the UNAIDS 90-90-90 targets. The HPTN 071 (PopART) study offered a unique opportunity to assess the performance of HIV-POCT conducted in the homes of over 17,000 participants in urban and peri-urban high HIV-burden communities in Zambia and

SA. Through comparison of results from field (household) HIV-POCT testing with laboratory-based testing on venous blood samples, we noted that despite careful and repeated user training and assessment and monitoring of cold chain storage of HIV-POCT kits, the sensitivity of field HIV-POCT is less than that reported for laboratory-based HIV testing [16].

The situation in the SA sites demonstrated a “perfect storm” of poor choice of HIV-POCT algorithms, inadequate QA and user error. It is impossible to identify which contributed most to the poor performance. The requirement for staff re-training to accommodate frequent changes in the type of HIV-POCT kits procured by SADOH is likely to have contributed to user error in this setting. Change in HIV-POCT kits to consistent use of a well-established algorithm in combination with strengthened training, supervision and quality management all played a part in improving the performance.

One critical stage in the performance of HIV-POCT is sample collection. This involves the use of different manufacturer-provided sample collection tools some of which are challenging for non-laboratory staff to use, for example, the capillary tube device. Additionally, some manufacturers offer complete kits but also sell the components individually which may result in HIV-POCT being conducted without the correct sample collection device. Panel proficiency testing does not test this step and whilst the use of dried samples, as is currently recommended by WHO for QA, allows for easier shipment of QA materials, it requires different skills in rehydration

and testing which do not reflect the real-life situation [7]. In the proficiency panel testing for this study with over 700 nurses and lay counsellors, the pass rate was consistently high (>95%), but user errors were detected when we implemented our increased supervision and use of a checklist (Appendix) which ensures that testers are assessed for proficiency in all stages of testing, including sample collection as well as counselling.

IQC of test kits after exposure to out-of-range temperatures in both countries did not reveal any functional abnormalities, suggesting that in this study, this factor did not contribute to the observed poor test kit performance. The number of test kits tested during internal QA was very large necessitating large quantities of positive and negative controls to be produced at a significant cost.

The laboratory GS used in this study included combined antigen–antibody 4th generation tests and viral load testing and so 3rd generation HIV-POCT will never be able to perform as well. However, it is unlikely that even with the anticipated differences in sensitivity between HIV-POCT 3rd generation antibody testing and laboratory testing, failure to identify acute infection was the primary driver of decreased sensitivity. Accounting for missed acute infections, which can be assumed to account for only a small proportion of the observed false negative HIV-POCT results, the performance of community-wide HIV-POCT was still not ideal. Laboratory testing, which was conducted during this study, is extremely labour-intensive and time-consuming and so it is not being recommended as an alternative to HIV-POCT. There is, however, a need to balance the widespread scale-up of HTS with quality of the results. Our results from the re-visits to participants with discrepant results in Zambia also show that laboratory testing may also have errors, possibly due to sample mislabelling.

The finding of increased false negative results in those individuals taking ART warrants further investigation. There is a paucity of evidence for decreased sensitivity of POCT in HIV-positive clients who are taking ART in the adult population; however, there is emerging evidence of this in children and adolescents [18,19]. HIV-POCT was not intended for use among individuals on ART, and this was an unexpected scenario in our study. In a “real-world” setting, this is a potentially important finding which requires further research and emphasizes the importance of appropriate messaging when offering community-based HIV testing, particularly with reference to limitation of HIV-POCT for individuals on ART. Further investigation of the association between ART exposure and false negative results and the possible immunological mechanisms underpinning this effect are outside the scope of this paper but should be a priority.

Few studies have been conducted comparing HIV-POCT using finger stick whole blood in field conditions with a laboratory GS. Specificity in data from the current study was high; we found very low levels of false positive rapid test results, in contrast to some studies [20]. Published data on sensitivity of HIV rapid tests in the field vary. One study from SA nested within the *Good Start Trial* showed sensitivity of 98% when comparing HIV-POCT tests with

laboratory-based HIV tests [10], whereas another South African study measured accuracy of HIV-POCT testing in a clinic setting and found high rates of false negative HIV tests (sensitivity 69%, 95% CI: 41–89%) which was improved by introduction of a different testing algorithm and QA measures [13]. The authors concluded that user error was the most significant contributor to inaccuracy.

Throughout the study period, the same HIV-POCT kits and QA/QC procedures were used for the CHiPs intervention as in the PC research cohort. Whilst parallel laboratory testing was not undertaken for the community members tested by CHiPs, we assume that similar challenges of HIV-POCT sensitivity are likely to have occurred in that context. Thus, it was critical to communicate the observed poor HIV-POCT performance to the community. Throughout the conduct of the HPTN 071 (PopART) study, the study team reported the findings of HIV-POCT performance to in-country ethics committees, study communities and international advisory boards, the study sponsor and Department of Health partners. In partnership with all stakeholders, community messaging was developed and delivered. This messaging focused on encouragement of repeat HIV testing for all at-risk individuals to avoid missed HIV diagnoses and consequently compromising individual health as well as risk of onward transmission and included reference to the fact that HIV rapid tests, like other diagnostic tests, are not 100% accurate.

Strengths and limitations

This study was conducted in the real-world setting using HIV-POCT as used in national algorithms and nationally approved QA procedures. The study setting offered a unique opportunity to compare HIV-POCT results to laboratory-based 4th generation testing completed in parallel on the same individuals. The study does, however, have limitations. It is difficult to attribute improvements in HIV-POCT sensitivity to specific factors, as multiple components of QA intervention were implemented concurrently with changes in test kits in SA. However, this is exactly how these changes would be implemented by national health systems. In the data shown here, the testing was conducted by nurses and we have assumed that similar results were seen in the HIV-POCT being done by lay counsellors at the same time using the same test algorithms and QA systems.

Conclusions

In conclusion, this is one of the first reports of wide-scale delivery of HIV-POCT in high-burden real-world settings compared to a laboratory GS. In this study, we demonstrate that detection of HIV infection can be improved significantly with enhanced user training, implementation of frequent and vigilant QA and QC monitoring and consistent use of an approved HIV-POCT algorithm. HIV RNA testing is more sensitive for detecting HIV infection than 4th generation assays but may not be feasible or affordable in some settings.

In order to reach our goals of universal knowledge of HIV status using large-scale non-facility-based HIV testing programmes, appropriate QA procedures must be carefully established and users must be adequately trained and

supervised in conducting all testing procedures. Programmes should also pay specific attention to advances in HIV-POCT technology and new evidence evaluating HIV-POCT in field settings, ensuring that they are using the best option for their setting.

Authors' affiliations

¹Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Stellenbosch University, Western Cape, South Africa; ²Zambart, University of Zambia, Lusaka, Zambia; ³Department of Pathology, HPTN Laboratory Center, Johns Hopkins University School of Medicine, Baltimore, MD, USA; ⁴Department of Clinical Research, London School of Hygiene and Tropical Medicine, London, UK; ⁵Statistical Center for HIV/AIDS Research and Prevention, Fred Hutchinson Cancer Research Center, Seattle, WA, USA; ⁶Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, UK; ⁷Science Facilitation Department, FHI 360, Durham, NC, USA; ⁸ICAP at Columbia University, Mailman School of Public Health, Columbia University, New York, NY, USA; ⁹Department of Medicine, Imperial College London, London, UK

Competing interests

No authors declare a conflict of interests.

Authors' contribution

PB, HA SF, RH, NB, DD, WES and EPW conceptualized the manuscript. MS, CP, KS, BK, NM, AJ, AB, AY and DD worked closely to develop the data set. All authors contributed towards development of the manuscript and reviewed drafts. All the authors have read and approved the final version.

Acknowledgements

The authors wish to acknowledge partners in Zambia and South Africa who have worked to implement the HPTN071/PopART trial and manage household rapid testing issues, notably Ministry of Health in Zambia and Department of Health in South Africa, the Zambian and South African community advisory boards and colleagues working for implementing partners (Kheth Impilo and ANOVA Healthcare in South Africa and FHI360 and Centre for Infectious Disease Research in Zambia). The authors also thank the study Data Safety and Monitoring Board and International Advisory Group for guidance with these issues as well as all study team members involved in the implementation of HPTN 071/PopART.

Funding

HPTN 071 is sponsored by the National Institute of Allergy and Infectious Diseases (NIAID) under Cooperative Agreements UM1-AI068619, UM1-AI068617 and UM1-AI068613, with funding from the U.S. President's Emergency Plan for AIDS Relief (PEPFAR). Additional funding is provided by the International Initiative for Impact Evaluation (3ie) with support from the Bill & Melinda Gates Foundation, as well as by NIAID, the National Institute on Drug Abuse (NIDA) and the National Institute of Mental Health (NIMH), all part of NIH. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIAID, NIMH, NIDA, PEPFAR, 3ie, or the Bill & Melinda Gates Foundation.

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Research article

Identification of misdiagnosed HIV clients in an Early Access to ART for All implementation study in Swaziland

Shaukat Khan^{1§}, Emma Mafara¹, Munyaradzi Pasipamire², Donna Spiegelman³, Sikhathale Mazibuko², Nombuso Ntshalintshali¹, Anita Hettema¹, Charlotte Lejeune¹, Fiona Walsh⁴ and Velephi Okello²

[§]Corresponding author: Shaukat Khan, Clinton Health Access Initiative (CHAI), 4th floor, Lilunga House, Somhlolo Rd, P.O. Box 6080, Mbabane, H100, Swaziland. (skhan@clintonhealthaccess.org)

Clinical Trial Number: NCT02909218

Abstract

Introduction: Rapid diagnostic testing has made HIV diagnosis and subsequent treatment more accessible. However, multiple factors, including improper implementation of testing strategies and clerical errors, have been reported to lead to HIV misdiagnosis. The World Health Organization has recommended HIV retesting prior to antiretroviral therapy (ART) initiation which has become pertinent with scaling up of Early Access to ART for All (EAAA). In this analysis, misdiagnosed clients are identified from a subgroup of clients enrolled in EAAA implementation study in Swaziland.

Methods: The subgroup to assess misdiagnosis was identified from enrolled EAAA study clients, who had an undetectable viral load prior to ART initiation between September 1, 2014 and May 31, 2016. One hundred and five of 2533 (4%) clients had an undetectable viral load prior to initiation to ART (pre-ART). The HIV status of clients was confirmed using the Determine HIV 1/2 and Uni-Gold HIV 1/2 rapid tests performed serially as recommended by the national testing algorithm. The status of clients on ART was additionally confirmed by fourth-generation HIV Ag/Ab combo tests, Architect and Genscreen Ultra.

Results: Fourteen of the 105 (13%) clients were false positive (HIV negative) on confirmation testing, of whom five (36%) were still in pre-ART care, while nine (64%) were in ART care. Overall, proportion of false positive was 0.6% (14/2533). The false-positive clients had a median CD4 of 791 cells/ml (interquartile range (IQR): 628, 967) compared to 549 cells/ml (IQR: 387, 791) for true positives (HIV positive) ($p = 0.0081$) and were nearly 20 years older ($p = 0.0008$).

Conclusions: Overall 0.6% of all enrolled EAAA clients were misdiagnosed, and 64% of misdiagnosed clients were initiated on ART. With adoption of EAAA guidelines by national governments, ART initiation regardless of immunological criteria, strengthening of proficiency testing and adoption of retesting prior to ART initiation would allow identification of misdiagnosed clients and further reduce potential of initiating misdiagnosed clients on ART.

Keywords: Early Access to ART for All; HIV misdiagnosis; HIV false positive; treatment for all; Universal test and treat; Swaziland; HIV testing

Received 16 December 2016; Accepted 25 June 2017; Published 29 August 2017

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Introduction

HIV is the leading public health concern in Swaziland with HIV prevalence of 32% and annual incidence of 2.4% among 18–49-year-old adults as determined by the 2011 Swaziland HIV Incidence Measurement Survey (SHIMS) [1]. SHIMS also identified substantial differences in prevalence among women (39%) and men (24%) and additionally reported that 38% of HIV-infected individuals were unaware of their HIV status [1]. The SHIMS data highlighted the need for an improvement of HIV testing and preventive care services in Swaziland. As effective HIV screening is critical for the identification of HIV-positive clients and their subsequent enrolment into antiretroviral therapy (ART) care, Swaziland has adopted the 2012 World Health Organization (WHO)-recommended HIV testing strategy for high-prevalence countries: the use of two rapid diagnostic tests (RDTs)

[2]. In Swaziland's national HIV testing algorithm, Alere Determine HIV-1/2 Ag/Ab Combo (Alere Inc., Yavne, Israel) is used as the first RDT and Uni-Gold HIV test (Trinity Biotech, Bray, Ireland) as the second RDT (Figure 1).

The RDT-based testing algorithm has been an essential tool for the diagnosis of HIV infection. In 2014 alone, the WHO reported that approximately 150 million individuals across 129 low- and middle-income countries have been tested for HIV, in large part due to availability of RDT [3]. The RDT-based testing algorithm has decentralized HIV testing services from laboratory settings to non-laboratory settings such as non-lab service points within facilities, community outreach campaigns and door-to-door testing. Although RDTs have been shown to be highly reliable for HIV diagnosis [4,5], recent reports have highlighted a substantial level of false-positive diagnoses in audits of testing programmes [3,6–8]. In addition, survey and

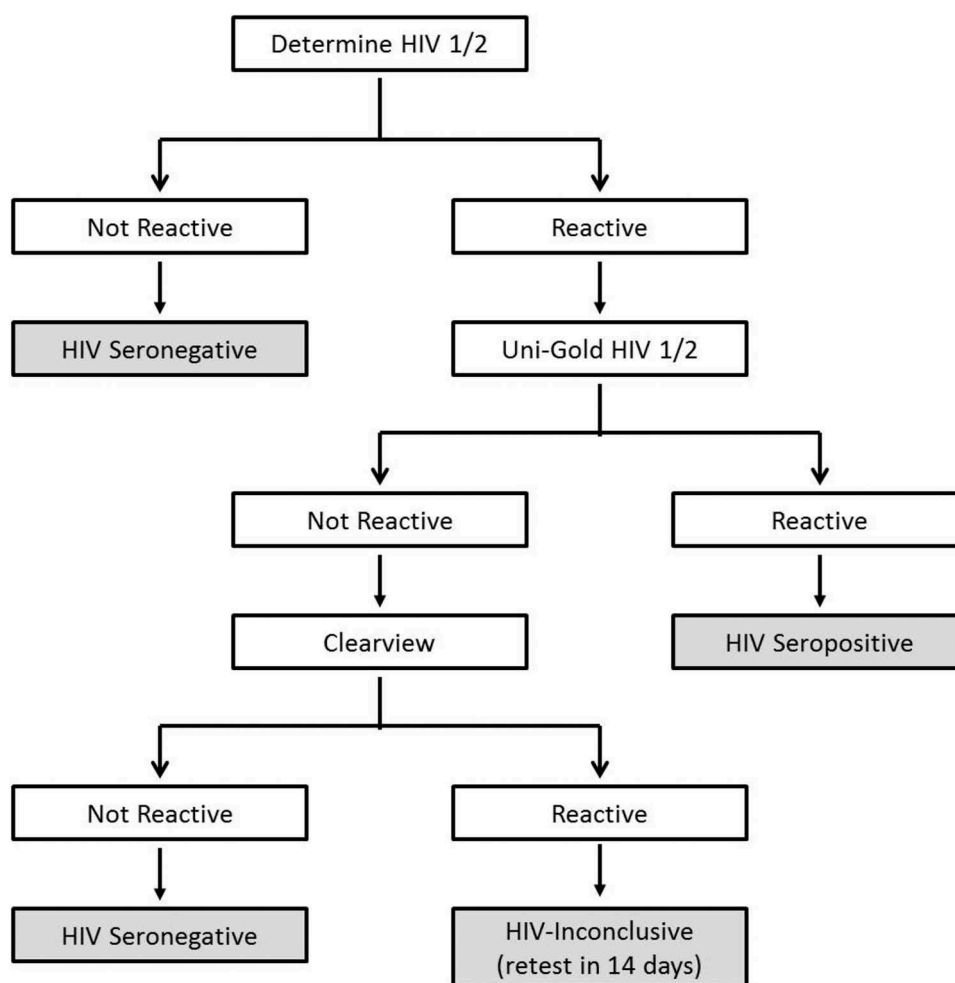


Figure 1. National testing algorithm.

The flow chart depicts Swaziland’s national testing algorithm for determination of HIV status. According to the national testing algorithm, a non-reactive first RDT is reported as HIV negative. In case of a reactive first RDT, a second RDT is conducted. If this second RDT is reactive, it is reported as HIV positive. However, if the result is non-reactive, a third RDT, Clearview COMPLETE HIV-1/2 (Chembio Diagnostic systems, Inc., Medford, NY, USA), is conducted. Non-reactive Clearview tests are reported as HIV negative while a reactive Clearview is reported as HIV inconclusive. Clients with inconclusive results are given another appointment to come after two weeks for a retest.

clinical trials have also identified false positives, albeit only in a subset of the retested samples [9–11].

In 2015, the WHO released new adult treatment guidelines that recommended offering ART as soon as possible following diagnosis [12]. As countries adopt these new guidelines, there is renewed scrutiny to ensure the validity of HIV testing programmes. Since 1997, WHO has been recommending retesting prior to ART initiation; however, a 2015 review of 48 national HIV testing policies found that only two programmes included testing before ART initiation in their national guidelines [13]. As of April 2017, Swaziland has not adopted retesting prior to ART initiation.

This analysis reports on the identification of misdiagnosed clients enrolled into an Early Access to ART for All (EAAA) implementation study.

Methods

Study population

The Maximizing Antiretroviral Therapy (ART) for better health and zero new infection (MaxART) EAAA implementation study was designed to determine the feasibility, acceptability, clinical outcomes, affordability and scalability of offering early antiretroviral treatment to all HIV-positive adults in Swaziland’s public sector health system. The trial uses a randomized stepped-wedge design across seven paired facilities (13 primary healthcare clinics and 1 regional hospital) in Hhohho Region. The trial includes all HIV-positive and self-reported ART-naïve adults ≥18 years of age, not pregnant or breastfeeding and able to give oral consent for an additional blood draw at enrolment and ART monitoring.

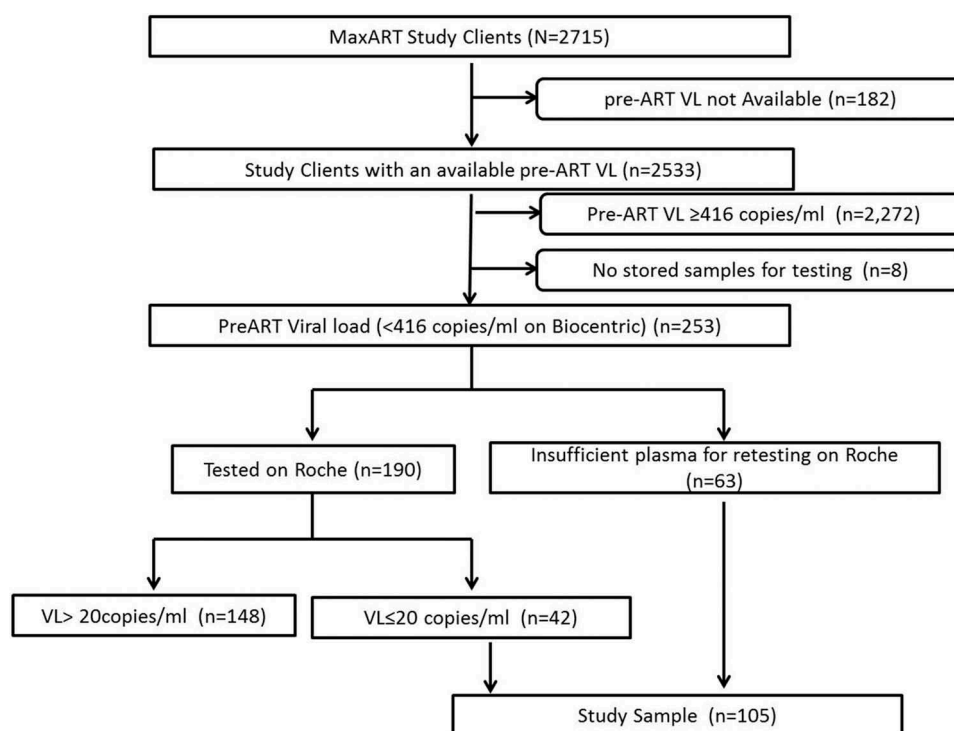


Figure 2. Procedure used to identify study sample for HIV status confirmation.

This flow chart depicts the procedures used to identify the sample population from the study population. The clients identified for HIV status confirmation testing were found to have an undetectable viral load on the Biocentric platform and/or on Roche platform at pre-ART enrolment.

Study sample

This paper describes the analysis of samples collected at the 14 study participation sites between September 1, 2014 and May 31, 2016. During this period, 2715 clients were enrolled in the study, of which 2533 (93%) had a pre-ART viral load (Figure 2). One sample of 6 ml of ethylenediaminetetraacetic acid (EDTA) anticoagulated blood was collected from each consenting client, which was used to prepare a plasma sample that was tested for viral load on the Biocentric platform (Bandol, France) (Instruments: Nordiag Arrow and Bio-Rad CFX 96 real-time detection Assay: Generic HIV Charge Virale). Any remaining plasma from this sample was then frozen at -80°C and stored at the National Reference Laboratory (NRL), Mbabane, Swaziland. Of 2533 clients, 253 (10%) of the clients had an undetectable pre-ART viral load and a stored frozen plasma sample (Figure 2). To exclude failure of viral RNA detectability due to the higher threshold of the Biocentric Platform (<416 copies/ml), samples were retested on the Roche Molecular Diagnostic viral load platform (Pleasanton, California, USA) (Instruments: COBAS AmpliPrep/COBAS Taqman System (CAP/CTM); Assay: CAP/CTM HIV-1 Test V2.0), which has a 20 copies/ml minimum detection threshold. As the Roche viral load protocol requires minimum of 1 ml plasma for testing, 190 of 253 (75%) samples were tested on the Roche platform (Figure 2) of which 42 of 190 (22%) samples were undetectable on Roche and were

included for confirmation of HIV Status. Remaining 63 of 253 (25%), which did not have sufficient sample for retesting on the Roche platform, were also included for confirmation of HIV status (Figure 2). A total of 105 of 2533 samples (4%) were identified for confirmation of HIV status (Figure 2).

National testing algorithm on frozen plasma samples

The clients' frozen plasma samples from the initial pre-ART viral load were retested at the NRL, using the Swaziland national testing algorithm, to determine HIV status (Figure 1).

Confirmation of HIV status

Clients testing non-reactive or indeterminate were requested for an additional blood draw. Two separate procedures were followed based on whether the client had been initiated on ART or not at the time of redraw.

For pre-ART clients, a fresh blood sample was drawn, and clients' HIV status was confirmed using the Swaziland national testing algorithm at the National Referral Laboratory (Figure 1). Clients who were dual reactive on Determine and Uni-Gold were interpreted as true positive (HIV positive) and non-reactive on Determine as false positive (HIV negative).

For ART clients, similar to pre-ART clients, a fresh blood sample was drawn, and clients' HIV status was determined

using the Swaziland national testing algorithm. As clients were on ART, additional testing was conducted using fourth-generation antigen/antibody (Ag/Ab) tests for HIV status confirmation. Fourth-generation tests, Abbott Architect HIV Ag/Ab combo assay (Abbott, Wiesbaden, Germany) and BioRad Genscreen Ultra HIV Ag/Ab combo assay (Bio-Rad, Marnes-la-Coquette, France) were conducted at the National Institute of Communicable Diseases in Johannesburg, South Africa. Clients who were dual reactive on the fourth-generation test were interpreted as true positive (HIV positive) and dual non-reactive as false positive (HIV negative).

Clinical characteristics of the clients

Clients' demographic and CD4 cell count results were obtained from the study database, which was extracted from the Ministry of Health's standard chronic care patient files as part of the EAAA study procedures.

Analysis

Statistical analysis was conducted using Stata version 12 (StataCorp, College Station, TX, USA).

Ethical considerations

The MaxART EAAA implementation study was approved by the Swaziland National Health Research Review Board in July 2014 (Reference Number: MH/599C/FWA 000 15267) and is registered on ClinicalTrials.gov with ID NCT02909218. Verbal consent was obtained from all study clients for collection and testing of blood samples in accordance with the approved protocol.

Results and discussion

Pre-ART viral load results were available for 2533 clients. Among those, 105 (4%) were below the limit of viral load detection on the Biocentric and/or Roche viral load platform. Previous studies have reported less than 1% of untreated HIV-positive individuals with an undetectable viral load [14,15]. However, the viral load platforms have become increasingly sensitive which could explain discrepancy in proportion of clients reported to be undetectable in previous studies and in current study. Even within this study, a difference in detectability due to threshold levels using Roche platform with 20 copies/ml versus Biocentric 416 copies/ml is evident.

Identifying potential misdiagnosed clients

Retesting of clients with an undetectable viral load resulted in confirmation of 88 of 105 (84%) clients as HIV positive. Sixteen of 105 (15%) clients were determined to be HIV negative and 1 (1%) client inconclusive, resulting in identification of 17 of 105 (16%) client for further HIV testing (Figure 3).

Confirmation of misdiagnosis

As initial testing was done on a frozen sample, a blood draw was requested from the 17 clients identified for further testing (Figure 3). At the time of the request for blood

draw, 7 of 17 (41%) clients were still in pre-ART care, while 10 of 17 (59%) had initiated ART.

Samples were redrawn from five of seven (71%) pre-ART clients, and all of them were false positive, according to the Swaziland national testing algorithm. Two (29%) pre-ART clients were lost to follow-up and were unable to be contacted for HIV status confirmation.

Samples from 9 of 10 (90%) ART clients were redrawn, and all of them were determined to be false positive, as they were HIV negative on repeat of the national testing algorithm on their pre-ART sample as well as on additional fourth-generation Ag/Ab. One (10%) ART client was unable to be contacted for redraw for confirmation testing as they were lost to follow-up.

Overall, 14 of 105 (13%) clients were identified as false positive. The false-positive ($n = 14$) clients represent 0.6% of the subset of all clients ($n = 2533$), which is considerably lower in comparison to 10% false-positive rate in Democratic Republic of Congo (DRC), 5% in Ethiopia and 3% in Burundi as determined from evaluation studies [6,7]. In Malawi, programmatic data have shown that 2% (547/30,300) of confirmatory tests could not be conclusively diagnosed [16].

Interviews with misdiagnosed ART clients

Follow-up interviews were conducted with the nine HIV-negative clients on ART to understand their HIV testing experience that led to them enrolling on ART. Six clients said they had tested HIV negative at the time of enrolment into care. Two clients were told that they were HIV positive yet did not see or have their results explained. One tested with their partner and they were shown one HIV-positive test result. These interviews highlighted several potential cofactors leading to their enrolment into care including administrative error, user error and clients' circumstantial belief that they are HIV positive.

Clinical characteristics of clients

The false-positive clients originated from 9 of 14 (64%) facilities (Table 1) with median age of 52 years old (interquartile range (IQR): 40, 63) compared to 35 years old (IQR: 28, 44) for true positives ($p = 0.0008$) and median CD4 at enrolment of 791 cells/ml (IQR: 628, 967) and 549 cells/ml (IQR: 387, 791) ($p = 0.0081$), respectively (Table 1). Mean difference in days between initial HIV-positive test and study enrolment for clients identified as false positive was 392 days (IQR: 45, 1587) compared to 201 days (IQR: 6, 981) for the true positives ($p = 0.2132$) (Table 1). Twenty-nine per cent of the false positive and 22% of true positive were male ($p = 0.584$) with 14% of the false positive single, 71% were married, while 41% of true positive were single and 53% were married at HIV diagnosis (Table 1).

This study found that clients with a false-positive result had significantly higher CD4 at enrolment than the true positives, 791 and 541 cells/ml, respectively ($p = 0.0081$), and there was a significant difference in median age among false positive and true positives, with the false positives being nearly 20 years older ($p = 0.0008$). The evaluation programmes in DRC reported a median age of 42 years old

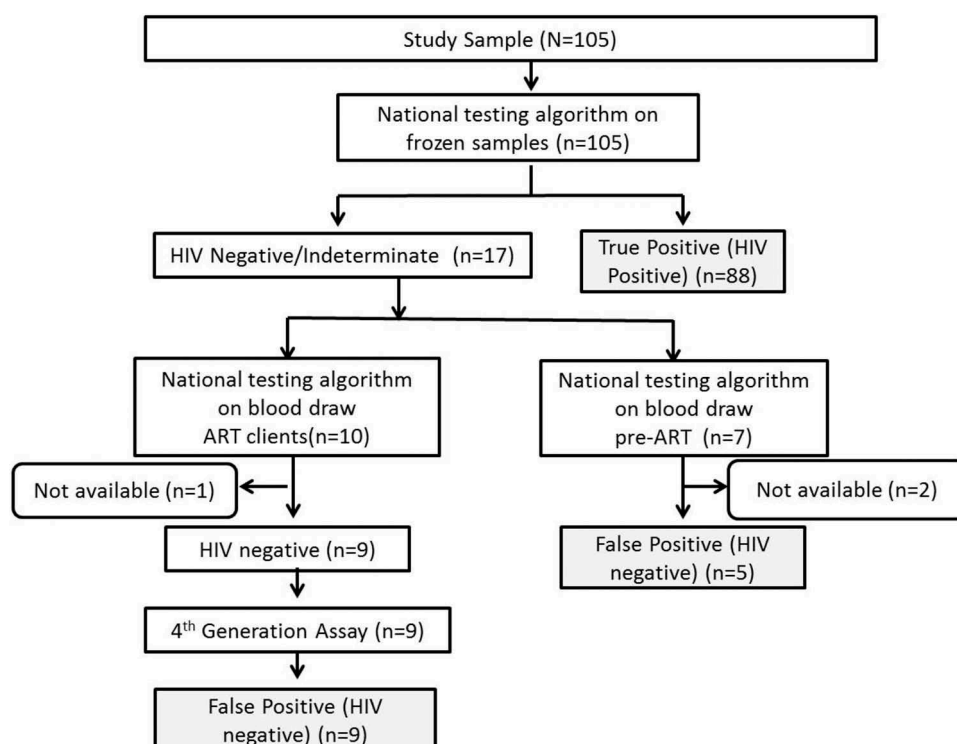


Figure 3. Testing procedure used to classify viral load undetectable clients as true positive (HIV positive) and false positive (HIV negative). Clients were classified as currently on ART or pre-ART at the time of their blood redraws. All clients with stored frozen samples prior to ART initiation were assayed using national testing algorithm. Clients who were determined to be HIV negative and/or indeterminate were requested for an additional blood draw to confirm their HIV status and the national testing algorithm was repeated on the blood redraws. If the client was on ART at the time of blood draw, the samples were further tested in parallel on Architect HIV Ag/Ab and Genscreen Ultra HIV Ag/Ab.

with median CD4 of 1107 cells/ml for false positives; however, there was no comparison statistics for true positive reported [7].

Limitations

The study was not designed to assess the validity of viral load as a diagnostic tool but as a selection tool to identify enrolled clients for investigation for HIV retesting. Our findings show a low proportion of false positives in a subset of clients enrolled in an implementation study over a period of 21 months. As the median time between initial test and enrolment of the study was 392 days (IQR: 45, 1587) and there is a lack of data from the time of the initial HIV test, we are unable to determine the cause of the misdiagnosis. However, systematic reviews have identified several potential causes of misdiagnosis [3,17]. In a WHO report on misdiagnosis, user error, suboptimal testing strategy, cross-reactivity and poor management, and supervision practices were suggested as factors related to less than optimal test specificity [3]. Additionally, programmatic data across different geographical location and time have shown variability in specificity of different RDTs, which can have an impact on false-positive rate [18]. In addition to specificity, low sensitivity of the RDTs has also been reported in field settings due to poor adherence to the recommended testing protocols [19].

Additional limitations of the study include the inability to identify the initial cause of undetectable pre-ART viral load in true positives. Due to lack of sufficient plasma, errors due to sample handling, plasma preparation, lab equipment error or user and administrative errors could not be ruled out. As the clients were self-reported ART naive, it was not possible to verify that they were virally suppressed in the absence of ART.

Enrolment of false positives in HIV care results in needless exposure to long-term ART that is detrimental to an individual's health and well-being and has potential adverse effects on relationships within their family and social circles. In addition, misdiagnosis creates undesirable wastage and unnecessary burden to the resources at the programmatic level, including health worker time and medical costs. Therefore, assuring and maintaining the quality of HIV testing services and consequently correct HIV diagnosis is an urgent priority, as even more intensive programmes are rolled out and more people are being offered immediate access to treatment without clinical or immunological threshold. Further studies are required to investigate quality of testing and accuracy of HIV testing in context of Swaziland to identify potential causes of misdiagnosis, such as user errors, inadequate training, interpretation of weak results, understanding and resolving of indeterminate

Table 1. Demographic and clinical characteristics of false and true positives

| Variable | | False positive (n = 14) | True positive (n = 91) | p-Value ^c |
|---|-----------------|-------------------------|------------------------|----------------------|
| Age | Median (Q1, Q3) | 52 (40, 63) | 35 (28, 44) | 0.0008 ^d |
| Sex | Male (%) | 4 (29) | 20 (22) | 0.584 ^e |
| CD4 ^a | Median (Q1, Q3) | 791 (628, 967) | 549 (387, 791) | 0.0081 ^d |
| Days from date of first reported HIV test and study enrolment | Median (Q1, Q3) | 392 (45, 1587) | 201 (6, 981) | 0.2017 ^d |
| Marital status ^b | Single (%) | 2 (14) | 36 (41) | 0.160 ^e |
| | Married (%) | 10 (71) | 46 (53) | |
| | Widowed (%) | 2 (14) | 4 (5) | |
| | Divorce (%) | 0 (0) | 1 (1) | |
| Facility (%) | | | | |
| Facility-1 | | 1 (7) | 4 (4) | 0.217 ^e |
| Facility-2 | | 1 (7) | 4 (4) | |
| Facility-3 | | 3 (21) | 8 (9) | |
| Facility-4 | | 2 (14) | 6 (7) | |
| Facility-5 | | 0 (0) | 4 (4) | |
| Facility-6 | | 2 (14) | 2 (2) | |
| Facility-7 | | 1 (7) | 10 (11) | |
| Facility-8 | | 1 (7) | 1 (1) | |
| Facility-9 | | 1 (7) | 8 (9) | |
| Facility-10 | | 0 (0) | 3 (3) | |
| Facility-11 | | 2 (14) | 14 (15) | |
| Facility-12 | | 0 (0) | 13 (14) | |
| Facility-13 | | 0 (0) | 12 (13) | |
| Facility-14 | | 0 (0) | 4 (4) | |

^aTen clients did not have an enrolment CD4 available.

^bFour clients did not have marital status recorded.

^cMissing values omitted in statistical analysis.

^dMann–Whitney test.

^e χ^2 -test.

results, adherence to testing procedures and workload. These studies in addition to the strengthening of existing proficiency testing and quality assurance systems to regulate and monitor performance of HIV testing are needed as even a small error rate can result in a high number of misdiagnosed cases in context of high testing volumes.

This is of particular importance as Swaziland and other sub-Saharan governments have embraced the new UNAIDS (90–90–90) targets [20]. To achieve the first 90, that 90% of people infected with HIV should know their status, governments will require not only increased testing but also innovative and smarter testing strategies. In addition to embracing the 90–90–90 targets, treatment for all is now a public health standard in most countries, including Swaziland which has adopted the 2015 WHO guidelines in October 2016.

Conclusions

The current findings showed an overall proportion of 0.6% false positives. With adoption of EAAA guidelines, ART initiation

regardless of immunological criteria, by national governments including Swaziland in October 2016, there is a need to strengthen national HIV testing processes including proficiency testing. In addition to improving HIV testing quality, adoption of retesting prior to ART initiation would also allow identification of clients misdiagnosed previously to further reduce potential of initiating misdiagnosed clients on ART.

Authors' affiliations

¹Clinton Health Access Initiative (CHAI), Mbabane, Swaziland; ²Department of Epidemiology, Ministry of Health, Mbabane, Swaziland; ³Harvard T.H. Chan School of Public Health, Boston, MA, USA; ⁴Clinton Health Access Initiative (CHAI), Boston, MA, USA

Competing interests

The authors declare that they have no competing interests.

Authors' contribution

SK designed the study, developed the methods, analysed the data and drafted the paper. EM, CL, FW, MP, SM, DS and VO designed the study and drafted the manuscript. AH and NN collected the data. All authors have read and approved the final version.

Abbreviations

ART, antiretroviral therapy; EAAA, Early Access to ART for All; MaxART, Maximizing antiretroviral therapy for better health and zero new infection; MoH, Ministry of Health; NRL, National Reference Laboratory; RDT, rapid diagnostic test; WHO, World Health Organization.

Acknowledgements

We would like to acknowledge Promise Dlamini, MoH Swaziland National ART Programme, for providing psychological support to study patients, Lungile Zwane for developing communication materials and Thomas How for reviewing the manuscript. We would also like to thank the Swaziland HIV Testing Services programme, Swaziland Health Laboratory Services, the EAAA study team, healthcare providers and patients at EAAA study sites for their tireless cooperation and patience during the study.

Funding

Mylan Laboratories Limited supported the study with donation of the anti-retroviral medication, Médecins Sans Frontières (with funding from UNITAID) with viral load testing and British Columbia Centre of Excellence in Canada with genotype testing. The study was financially supported by the Dutch Postcode Lottery in the Netherlands, and the Embassy of the Kingdom of the Netherlands in Mozambique.

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Research article

Misdiagnosis of HIV infection during a South African community-based survey: implications for rapid HIV testing

Tendesayi Kufa^{§1,2}, Ayesha BM Kharsany³, Cherie Cawood⁴, David Khanyile⁴, Lara Lewis³, Anneke Grobler³, Zawadi Chipeta⁵, Alfred Bere⁵, Mary Glenshaw⁵ and Adrian Puren^{1,6}

[§]**Corresponding author:** Tendesayi Kufa, Centre for HIV and STIs, National Institutes of Communicable Diseases, 1 Modderfontein Road, Sandringham South Africa. Tel: +27 11 555 0475. (tendesayik@nicd.ac.za; tendesayik@yahoo.com)

Abstract

Introduction: We describe the overall accuracy and performance of a serial rapid HIV testing algorithm used in community-based HIV testing in the context of a population-based household survey conducted in two sub-districts of uMgungundlovu district, KwaZulu-Natal, South Africa, against reference fourth-generation HIV-1/2 antibody and p24 antigen combination immunoassays. We discuss implications of the findings on rapid HIV testing programmes.

Methods: Cross-sectional design: Following enrolment into the survey, questionnaires were administered to eligible and consenting participants in order to obtain demographic and HIV-related data. Peripheral blood samples were collected for HIV-related testing. Participants were offered community-based HIV testing in the home by trained field workers using a serial algorithm with two rapid diagnostic tests (RDTs) in series. In the laboratory, reference HIV testing was conducted using two fourth-generation immunoassays with all positives in the confirmatory test considered true positives. Accuracy, sensitivity, specificity, positive predictive value, negative predictive value and false-positive and false-negative rates were determined.

Results: Of 10,236 individuals enrolled in the survey, 3740 were tested in the home (median age 24 years (interquartile range 19–31 years), 42.1% males and HIV positivity on RDT algorithm 8.0%). From those tested, 3729 (99.7%) had a definitive RDT result as well as a laboratory immunoassay result. The overall accuracy of the RDT when compared to the fourth-generation immunoassays was 98.8% (95% confidence interval (CI) 98.5–99.2). The sensitivity, specificity, positive predictive value and negative predictive value were 91.1% (95% CI 87.5–93.7), 99.9% (95% CI 99.8–100), 99.3% (95% CI 97.4–99.8) and 99.1% (95% CI 98.8–99.4) respectively. The false-positive and false-negative rates were 0.06% (95% CI 0.01–0.24) and 8.9% (95% CI 6.3–12.53). Compared to true positives, false negatives were more likely to be recently infected on limited antigen avidity assay and to report antiretroviral therapy (ART) use.

Conclusions: The overall accuracy of the RDT algorithm was high. However, there were few false positives, and the sensitivity was lower than expected with high false negatives, despite implementation of quality assurance measures. False negatives were associated with recent (early) infection and ART exposure. The RDT algorithm was able to correctly identify the majority of HIV infections in community-based HIV testing. Messaging on the potential for false positives and false negatives should be included in these programmes.

Keywords: HIV; antibody testing; sensitivity; specificity; misdiagnosis

Received 15 December 2016; Accepted 19 June 2017; Published 29 August 2017

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Introduction

HIV counselling and testing (HCT) is the gateway to care and treatment, including antiretroviral therapy (ART), for HIV-positive patients [1]. The widespread use of rapid diagnostic tests (RDTs) in high HIV prevalence settings has resulted in the increase in the number of people tested for HIV and the decentralization of HIV testing from health facilities into communities, reaching more young people, males, first-time testers and those at higher CD4 cell counts, and HIV-related less morbidity [2,3]. For example, 13.3 million people were tested for

HIV in a national HIV testing campaign conducted in South Africa in the period 2011 to 2012 [4,5], and it is estimated that 9.9 million individuals were tested in 2015 [6]. With the introduction of the universal test and treat strategy and antiretroviral (ARV)-based HIV prevention strategies such as pre-exposure prophylaxis (PrEP), more and more people will be expected to test for HIV on a regular basis in order to initiate ART immediately or continue taking PrEP [7]. The need for HIV services to provide accurate HIV test results can therefore not be overstated.

HIV misdiagnosis occurs when an HIV-uninfected individual is incorrectly classified as HIV infected by the test used or vice versa [8]. There are multiple factors which can cause or contribute to HIV misdiagnosis. These vary from suboptimal testing strategies (including poor selection of assays used to construct algorithms and use of tiebreakers), deviation from standardized testing algorithms, user errors such as incorrectly performing test procedures, incorrectly interpreting test results, non-adherence to testing standard operating procedures as well as clerical errors [8–13]. False-positive rates as high as 10.3% upon retesting have been observed in some settings [14]. The consequences of HIV misdiagnosis are serious. False-positive HIV test results can result in the unnecessary treatment of HIV-uninfected individuals as well as exposure to the psychological trauma and stigmatization that may be associated with a diagnosis of HIV infection and the loss of credibility by HIV testing programmes [15]. On the other hand, false-negative HIV test results represent missed opportunities for entry into HIV care and treatment and the risk of unknowingly transmitting HIV to uninfected partners.

To reduce the risk of HIV misdiagnoses, the World Health Organization recommends the use of approved testing algorithms as well as the implementation HIV rapid test quality assurance programmes [8]. Key facets of these programs include training, retraining and mentoring of testing personnel, developing standardized registers to document HIV testing results, strengthening supply chains for RDT, developing standard operating procedures for rapid HIV testing, implementing internal and external quality controls, retesting and external quality assessments and proficiency testing as well as continuous monitoring and evaluation of these programmes [8]. These measures are essential especially for community-based testing programs where HIV testing may occur under less-than-ideal conditions with respect to the environmental temperatures at which RDT kits may be stored while in the field and the high volume of tests conducted. HIV testing in these settings is mostly conducted by community health workers who are generally well trained and highly proficient in HIV testing. However, in a few instances, lower accuracy has been documented among community workers compared to laboratory staff [16]. The performance of rapid HIV testing has also been found to vary with the reference standard used for evaluation. RDTs are second- or third-generation tests capable of detecting HIV-1 envelope protein antibodies, while fourth-generation tests are capable of detecting both antibodies to envelope proteins and p24 antigens [17–19]. The fourth-generation tests have been found to have fewer false positives and false negatives and should be better able to detect HIV infections earlier than third-generation tests [13]. We describe the overall accuracy and performance of the nationally recommended serial RDT algorithm against the nationally recommended laboratory-based fourth-generation immunoassays (IAs) in a household HIV prevalence survey during which rapid HIV testing was offered to willing and consenting participants. We discuss the implications of the findings for community-based HIV testing.

Methods

Study design and setting

Data used in this study were collected during a cross-sectional, household survey conducted in the Vulindlela and Greater Edendale sub-districts of uMgungundlovu district, KwaZulu-Natal, South Africa, during the period July 2015 to May 2016. This household survey was the second survey on the HIV Incidence Provincial Surveillance System (HIPSS) platform initiated in 2014 with the aim of establishing population-level estimates of HIV incidence and prevalence in the two sub-districts. The methods of the studies on this platform have been previously described [20]. Briefly, the HIPSS platform consists of two sequential cross-sectional household surveys conducted 1 year apart, each with approximately 10,000 individuals in the age group 15–49 years, residing in the Vulindlela and Greater Edendale sub-districts. Individuals were randomly selected from eligible households which in turn had been randomly selected from randomly selected census enumeration areas.

Data collection procedures

Following eligibility assessment and informed consent procedures, eligible and willing individuals were enrolled into the second survey. A questionnaire was administered by trained field workers using personal digital assistants. Data on demographic, socio-economic and behavioural characteristics were collected as were data on access to HIV testing, care and treatment. Field workers then collected 25 ml whole blood specimens for HIV and related testing in the laboratory. Participants were offered field worker provided, rapid HIV testing in the home and referred to the local clinic for HIV care and treatment if the HIV result was positive. Field workers also completed a paper-based laboratory tracking form in which they documented rapid HIV test results in addition to other specimens collected.

Rapid HIV testing and quality assurance

Figure 1 shows the HIV testing algorithms used for the community-based rapid testing and the reference fourth-generation IAs used in the laboratory. The rapid HIV testing algorithm used two RDTs in a serial algorithm: blood specimens collected by finger prick were tested with the first rapid test (RDT 1 - Alere Determine HIV-1/2, Matsudo, Japan), and if the test was reactive, a second rapid test (RDT 2 - UniGold HIV, Trinity Biotech, Bray, Ireland) was used to confirm the first HIV-reactive result. If RDT 2 was also reactive, the participant was considered HIV positive, received post-test counselling and referred to a local clinic for HIV care and treatment. If the RDT 1 was non-reactive, the participant was considered HIV negative and counselled on staying negative with and no further testing in the home. If the RDT 2 was non-reactive, the participant had a discrepant HIV result and was not given a result but was informed that the team will return with an HIV result once the laboratory testing was completed. The field workers were trained to conduct the rapid testing according to the manufacturer's instructions, which for RDT 1 (Alere Determine HIV) meant collecting 50 µl of whole blood via finger prick, applying specimen to absorbent pad on the

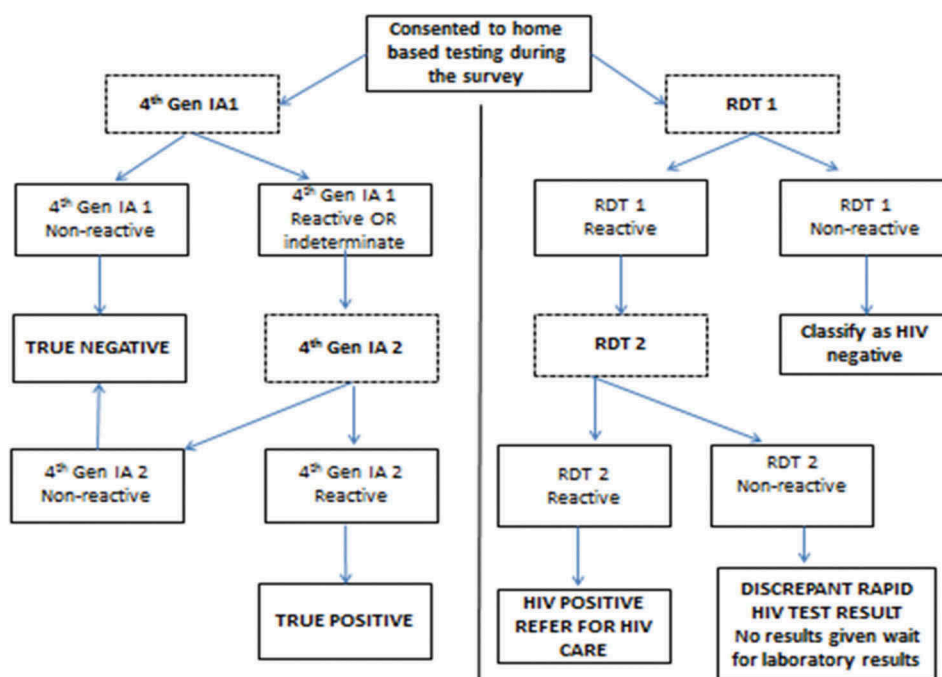


Figure 1. HIV testing algorithms.

RDT 1 - Alere Determine HIV-1/2 (Matsudo, Japan), RDT 2 - UniGold HIV (Trinity Biotech, Bray, Ireland), 4th Gen IA1 - Vironostika HIV Uniform II antigen/antibody (Biomerieux, The Netherlands), 4th Gen IA2 - The Elecsys HIV Combi PT 4th Gen Assay, Roche Diagnostics, GmbH (Penzberg, Germany).

test strip, adding one drop of chase buffer and waiting 15 min (mechanically timed) before reading the result. For RDT2 (UniGold HIV), this meant applying two drops of blood specimen to the sample port, followed by two drops of wash reagent and waiting 10 min (also mechanically timed) before reading results. The algorithm used during the survey differed slightly to the nationally recommended algorithm [21], which at the time recommended the use of Advanced Quality Rapid anti-HIV [1,2] test (InTec Products INC) as a screening test with non-reactive results considered as HIV negative, and any reactive results confirmed with Abon HIV 1/2/0 Tri-Line Rapid test kit (Abon BioPharm). In the case of discrepant results, national algorithm recommended repeating the test with both the screening and confirmatory tests, and if still not resolved that, a blood specimen be collected for ELISA testing in the laboratory [21].

At appointment to the survey, the field workers who conducted the community-based HIV testing had an HCT certificate in accordance with National Department of Health guidelines. As part of the survey protocol training, they received an additional 2 days' refresher training covering counselling and communication, national testing algorithms, rapid testing using survey-specific test kits, referrals and linkage into care and proficiency testing. Biweekly proficiency testing for the field workers was conducted throughout the survey with provision for retraining provided for those who failed it. The proficiency testing conducted at local field offices involved the laboratory sending

specimen panels to the field workers to test and return results for comparison. Field supervisors also conducted random checks and shadowed home visits to ensure adherence to standard operating procedures.

Laboratory HIV testing

Laboratory-based HIV testing used two fourth-generation IAs also in series. Participants' blood specimens were first tested with the first IA (Vironostika HIV Uniform II antigen/antibody (Biomerieux, The Netherlands)), and if reactive (cutoff value = mean of three negative controls + 0.1), a second assay (The Elecsys® HIV Combi PT 4th Gen Assay, Roche Diagnostics, GmbH, Penzberg, Germany) was used to confirm the HIV reactive. The cutoff indices for the Elecsys® HIV Combi PT assay were as follows: 0.00–0.90 - non-reactive; 1.00–2.0 - weakly reactive/borderline; >2.0 - reactive. Following the manufacturer's discontinuation of production of the Vironostika HIV Uniform II antigen/antibody assay, a small proportion of the samples (32 out of 10,236 tested for HIV in the laboratory (0.3%)) were tested using the Elecsys® HIV Combi PT 4th Gen Assay as the screening assay, and reactive results were confirmed using the Siemens Advia Centaur HIV Ag/Ab Combo (CHIV) assay (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA). Cutoff index value of 1.0 was used to determine whether a specimen was reactive or non-reactive. All HIV-positive results were further confirmed by Western blotting and HIV viral load testing. Participants whose confirmatory (second) IA was reactive were considered true positives while

those whose initial screening test was non-reactive or their confirmatory test was non-reactive, true negatives. Where the laboratory-based results and the community-based RDT results were discrepant, participants were informed of the laboratory-based results. In addition, if the RDT1 and RDT2 results were discrepant, participants were also informed of the laboratory-based results. Limiting antigen avidity enzyme immunosorbent assay (LAg Avidity EIA) testing was undertaken on all EIA antibody-positive samples to determine recent (early) HIV infection.

Variables and outcomes

The main outcome of the study was the accuracy of the RDT algorithm, and this was defined as the overall proportion of individuals tested with an HIV RDT in the home who had the correct HIV result on the reference fourth-generation IA. Other outcomes determined were the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), false-positive rate and false-negative rate of the HIV RDT algorithm compared to the reference standard of laboratory-based IA algorithm. This reference standard was chosen to match the reference standard for HIV testing in the South African national HIV testing programme [21]. As the RDTs used in the survey were antibody-only HIV testing assays and were expected to be less sensitive compared to fourth-generation IA that detect for antibody and p24 antigen, their performance against an alternative reference standard, Western blot assay (New LAV Blot 11 Western blot assay, Bio-Rad, France), was also evaluated.

Data analysis and statistical methods

The population tested by HIV RDT was described using descriptive statistics - median and interquartile ranges (IQR) for continuous variables as well as counts and proportions for categorical variables. The outcomes as described were determined as proportions with Wilson's binomial 95% confidence intervals (CIs) calculated around the estimates. In order to assess any potential effects of immunological and virological status on the sensitivity of RDT, participants who tested false negative on RDT were compared to the true positives who were correctly diagnosed by RDT with respect to median CD4 cell count at enrolment, median viral load and proportion with viral loads >1000 copies/ml. The Wilcoxon rank sum and Chi-squared tests were used to assess statistically significant differences between these groups with p -values <0.05 considered statistically significant.

Ethical considerations

The study was approved by the University of KwaZulu-Natal's Biomedical Research Ethics Committee, the Centers for Disease Control and Prevention (CDC) Office of the Associate Directors of Science. Permissions to conduct the study were granted by the KwaZulu-Natal Provincial Department of Health and the uMgungundlovu District Municipality. Verbal informed consent was obtained from the head of the household and written informed consent obtained from the eligible individuals who were 18 years of age and older. For minors aged 15–18 years, written informed consent was obtained from the parents,

guardians or caregivers and an individual assent obtained from the minor.

Results

Description of the community-based testing programme

A total of 50 field workers were trained and conducted the community-based rapid HIV testing. During the survey, none of the field workers failed proficiency testing, and there were no errors documented during proficiency testing or field supervision visits, although the documentation of proficiency testing and user errors was inconsistent and sometimes incomplete.

Characteristics of participants included in the analysis

A total of 10,236 individuals were enrolled into the survey. Of these, 6389 (62.5%) did not consent to rapid HIV testing, while an additional 107 (1.0%) consented but ended up not testing. Of all the 6496 who were not tested, 5905 (90.9%) reported prior HIV testing, with 2749 (46.6%) self-reporting an HIV-positive status. The most common reason provided for declining an HIV test was prior knowledge of HIV status (4788 - 73.7%) and being afraid to test (1050 - 16.6%). In total, there were 3740 participants who were tested by RDT (see Figure 2). Table 1 shows the demographic and social characteristics of the participants who tested. The median age was 24 years (IQR 19–31 years), 1573 (42.1%) were males, 3092 (82.7%) were single, never been married or lived as married, and 3142 (84.0%) had been tested for HIV prior to the survey.

Rapid HIV testing results

Of the 3740 participants tested with RDT 1, 315 (8.4%) were reactive and were eligible for testing using RDT 2. Of these, 11 were not tested with RDT 2 (reasons not provided), 300 were reactive on RDT 2 and 4 were non-reactive and therefore had discrepant RDT results (Figure 2). The prevalence of discrepant rapid HIV test results was 0.1% among all those tested by RDT and 1.3% among those who tested positive on RDT 1. Of the four with discrepant results, two (50%) were subsequently confirmed to be HIV positive with the laboratory IA testing algorithm.

Performance of the RDT compared to laboratory-based IA tests

Among the 3740 participants tested using the RDT algorithm, there were 3708 (99.1%) who had a definitive result on the RDT algorithm and were tested for HIV using the fourth-generation IA algorithm in the laboratory; excluding 11 not tested by RDT, 4 with discrepant RDT results and 11 who were not tested by fourth-generation IA in the laboratory (1 RDT-positive and 10 RDT-negative reasons not stated) and 6 who had discrepant EIA results (Figure 2). Of the 3708, 326 (8.8%) were reactive on the laboratory fourth-generation EIA algorithm and therefore considered the true HIV positives, while 3382 (91.2%) were non-reactive on the laboratory fourth-generation EIA and considered true HIV negatives. Of the true negatives, two (0.06%; 95% CI 0.02–0.22) false positives were identified. Among the 326 true HIV positives, 29 (8.9%; 95% CI 6.3–12.5) had tested HIV

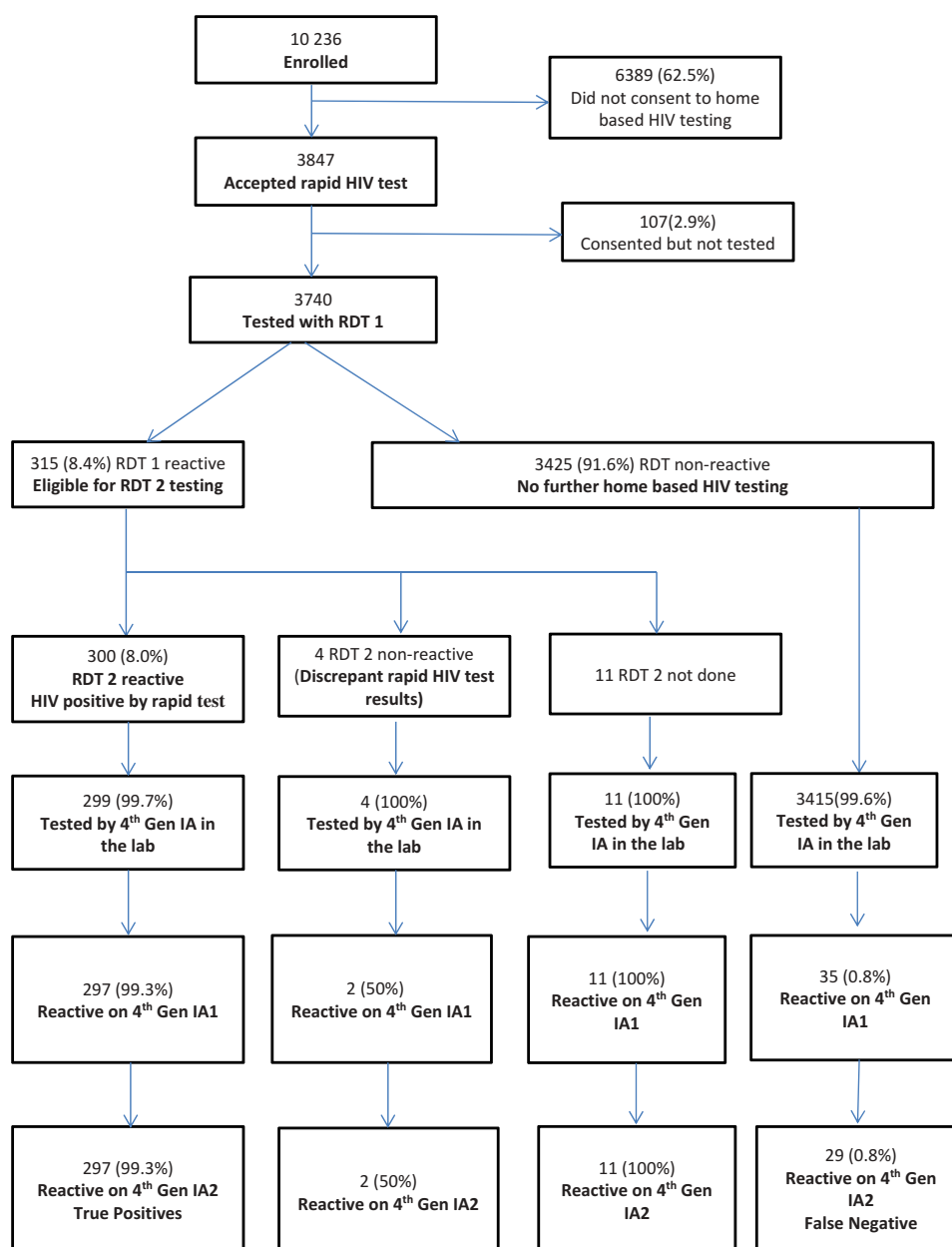


Figure 2. Study flow.

negative by RDT algorithm at home and were therefore false negatives (see Table 2). The overall accuracy of the RDT algorithm in the home-based testing was 98.8% (95% CI 98.5–99.2). However, the sensitivity of the RDT algorithm in this setting was lower than expected at 91.1% (95% CI 87.5–93.7). The specificity of the RDT algorithm was 99.9% (95% CI 99.8–100). The positive and negative predictive values were 99.3% (95% CI 97.6–99.8) and 99.1% (95% CI 98.8–99.4). When compared against the fourth-generation EIA as a reference standard and Western blot (which identified 323 HIV-positive individuals compared to 326 using fourth-generation testing alone), the sensitivity, specificity, PPV and NPV of RDTs were 92% (95% CI 88.5–94.4), 99.9%

(95% CI 99.8–100), 99.3% (95% CI 97.4–99.8) and 99.2% (95% CI 98.9–99.5) respectively. The false positives and false negatives were 0.06% (95% CI 0.02–0.22) and 8.0% (95% CI 5.6–11.5) respectively.

In an analysis excluding 13 individuals ($N = 3695$) who self-reported being HIV positive and were tested by both RDT in the home and EIA in the lab, the sensitivity, specificity, PPV and NPV of RDTs were 91.7% (95% CI 88.1–94.3), 99.9% (95% CI 99.8–100), 99.3% (95% CI 97.3–99.8) and 99.2% (95% CI 98.9–99.5), respectively. The false-positive and false-negative rates unchanged at 0.06% (95% CI 0.02–0.24) and 8.3% (95% CI 5.7–11.9), respectively.

Table 1. Characteristics of participants who tested by rapid diagnostic test (RDT) in the home (N = 3740)

| Variables | |
|--|-------------|
| Age (median, IQR) in years | 24 (19–31) |
| Males (n, %) | 1573 (42.1) |
| Black African (n, %) | 3726 (99.6) |
| Completed 12 or more years of schooling (n, %) | 1726 (46.1) |
| Single (never been married OR cohabited) (n, %) | 3092 (82.7) |
| Perceived themselves to be at risk of HIV infection (n, %) | 1436 (38.4) |
| Previous HIV testing (n, %) | 3142 (84.0) |
| Self-reported being HIV positive | 14 (0.5) |
| Self-reported taking ART at enrolment (n, %) | 3 (0.1) |
| Tested HIV positive in the home | 300 (8.0) |
| Final HIV-positive status ^a | 339 (9.1) |

^aOut of 3729 as 11 tested by RDT in the home not tested by laboratory-based IA.

IQR: interquartile range; ART: antiretroviral therapy.

Description of the false positives

There were two individuals who were falsely positive on RDT. Both individuals were female, were not pregnant, were not taking any medications and did not suffer from chronic illnesses at the time of enrolment. These two individuals both reported testing HIV negative within the preceding 90 days. There were no obvious reasons to explain the false-positive results. However, clerical errors cannot be excluded.

Comparison of virological and immunological profiles of false negatives to true positives

HIV-positive individuals who were incorrectly diagnosed as HIV negative by the RDT (false negatives) were not

significantly different from those who were correctly diagnosed as HIV positive by RDT with respect to proportions who had detectable HIV RNA and median viral load (see Table 3). There was also no difference in the median CD4 counts (557 cells/ μ l (IQR 200–753 cells/ μ l)) vs. 430 cells/ μ l (266–610 cells/ μ l), $p = 0.380$) and median CD4: CD8 ratios (0.5 (95% CI 0.2–0.9) vs. 0.4 (95% CI 0.2–0.6), p -value 0.2) among the false negatives. False-negative individuals were also more likely to be LAg avidity EIA positive (27.6% vs. 7.4%, $p = 0.001$), to report being HIV positive (10.3% vs. 2.7%, $p = 0.006$) and taking ART at enrolment (3.5% vs. 0.3%, $p = 0.170$) although the latter represented only one individual in each group. Among the false negatives were two individuals who met criteria for acute HIV infection (Western blot negative or indeterminate AND detectable viral load) accounting for only 0.6% of all true HIV positives and 6.9% of the false negatives.

Discussion

We describe the performance of RDT in a serial algorithm used for community-based HIV testing during a household survey to measure HIV prevalence and incidence. In this setting, the overall accuracy of the RDT algorithm compared to a reference standard of fourth-generation laboratory-based IAs was high at 99.0%, but sensitivity was lower than the WHO-recommended level of $\geq 99\%$ at 91.1% with a false-negative rate of 8.9%. Participants incorrectly diagnosed as HIV negative by the RDT algorithm did not differ significantly from those correctly diagnosed as HIV positive with respect to CD4 cell counts, CD8 cell counts, CD4:CD8 ratios and median viral loads among those with detectable virus, although were more likely to be classified as recently infected by the LAg assay and to self-report being HIV positive. There was a low false-positive rate at 0.06%. The performance of the RDT was similar when comparing the fourth-generation IAs and Western blot (equivalent to the third-generation HIV testing) as reference methods.

Table 2. Performance of home-based rapid diagnostic test (RDT) compared to fourth-generation immunosorbent assay (EIA) and to fourth-generation EIA and Western blot (N = 3708)

| Parameters | Fourth-generation immunoassay | | Fourth-generation immunoassay and Western blot | |
|---------------------------|-------------------------------|------------------|--|------------------|
| | n/N | % (95% CI) | n/N | % (95% CI) |
| Accuracy | 3677/3729 | 98.8 (98.5–99.2) | 3680/3708 | 99.2 (98.1–99.5) |
| Sensitivity | 297/326 | 91.1 (87.5–93.7) | 297/323 | 92 (88.5–94.4) |
| Specificity | 3380/3382 | 99.9 (99.8–100) | 3383/3385 | 99.9 (99.8–100) |
| Positive predictive value | 297/299 | 99.3 (97.6–99.8) | 297/299 | 99.3 (97.6–99.8) |
| Negative predictive value | 3380/3409 | 99.1 (98.8–99.4) | 3383/3409 | 99.2 (98.9–99.5) |
| False-positive rate | 2/3382 | 0.06 (0.02–0.22) | 2/3385 | 0.06 (0.02–0.22) |
| False-negative rate | 29/326 | 8.9 (6.3–12.5) | 26/323 | 8.0 (5.6–11.5) |

RDT 1 - Alere Determine HIV-1/2 (Matsudo, Japan); RDT 2 - UniGold HIV (Trinity Biotech, Bray, Ireland), 4th Gen IA1-Vironostika HIV Uniform II antigen/antibody (Biomerieux, The Netherlands), 4th Gen IA2 - The Elecsys® HIV Combi PT 4th Gen Assay, Roche Diagnostics, GmbH (Penzberg, Germany), Western blot assay - New LAV Blot 11 Western blot assay (Bio-Rad, France).

CI: confidence interval.

Table 3. Immunological and virological profiles of false negatives compared to true positives

| Variables | HIV positive (false negatives) (N = 29) | HIV positive (true positives) (N = 297) | p-Value |
|---|--|--|--------------------|
| Detectable viral load (viral load >20 copies/ml) (n, %) | 26 (89.7) | 267 (89.9) | 0.967 ^a |
| HIV RNA viral load, copies/ml (median, IQR) | 17,000 (5600–54,000) | 24,000 (3700–100,000) | 0.679 ^c |
| Viral load >1000 copies/ml, (n, %) | 23 (79.3) | 246 (82.8) | 0.634 ^a |
| CD4 cell count (median, IQR) cells/μl | 557 (200–753) | 430 (266–610) | 0.308 ^c |
| CD8 cell count (median, IQR) cells/μl | 880 (750–1346) | 990 (776–1343) | 0.487 ^c |
| CD4:CD8 ratio (median, IQR) | 0.5 (0.2–0.9) | 0.4 (0.2–0.6) | 0.2 ^c |
| LAg avidity EIA positive (n, %) | 8 (27.6) | 22 (7.4) | 0.001 ^b |
| Western blot negative or indeterminate (n, %) | 3 (10.4) | 0 (0.0) | 0.001 ^a |
| Acute infection (n, %) | 2 (6.9) | 0 (0.0) | 0.001 ^a |
| Self-reported being HIV positive (n, %) | 3 (10.3) | 8 (2.7) | 0.064 ^a |
| Self-reported taking ART (n, %) | 1 (3.5) | 1 (0.3) | 0.170 ^a |

^aFisher's exact Chi-squared test.

^bChi-squared test.

^cWilcoxon rank sum test *p*-value.

LAg avidity EIA: limiting antigen avidity enzyme immunosorbent assay; IQR: interquartile range; ART: antiretroviral therapy.

The low sensitivity of the RDT in community-based testing was unexpected and concerning. Several studies of RDT performance in both community- and health facility-based HIV testing have reported higher sensitivities than observed in our study. Molesworth et al. found a sensitivity of 99% comparing third-generation RDT kits with third-generation EIA during HIV testing in the context of a household survey, using initially two RDTs in parallel with a tiebreaker, then two RDTs in series with a tiebreaker and finally retesting all positives and 10% of the negatives in the laboratory [22]. Jackson et al. found a sensitivity of 98% in a community randomized controlled trial of home-based HCT conducted by lay counsellors again comparing third-generation RDT kits with a third-generation EIA [23]. Wolpaw et al. found an RDT sensitivity of 68.7% which improved to 93.5% following switching test kit brands and to 98% following implementation of quality improvement measures upon retesting individuals who previously tested HIV negative at primary care clinics in Cape Town, South Africa [24]. In this Cape Town study, the inconsistent use of chase buffer and early reading of results were common errors observed and targeted for quality improvement interventions [24].

This reduced sensitivity has wide-ranging implications for HIV prevention, care and treatment in South Africa. A false-negative result may result in inadvertent transmission of HIV to uninfected partners by individuals who believe they are HIV negative. With the rollout of PrEP among men-who-have-sex-with-men and female sex workers [25], a false-negative diagnosis implies continuing with PrEP when a full treatment regimen is required which may lead to ARV drug resistance. With the implementation of universal test and treat, a false-negative diagnosis may also result in delayed entry into care, resulting in excess morbidity and mortality from HIV.

A number of factors could explain the lower sensitivity of the RDT algorithm observed in our survey. The use of fourth-generation EIA has been found to have fewer false

positives and false negatives and able to detect more acute infections compared to third-generation tests [13]. However, the relatively low proportion of false-negative individuals who had acute HIV infection (10.4% of true positives and 0.09% of RDT negatives) suggests that this was unlikely to be a main factor contributing to the high false-negative rate. These rates of acute infections observed in our study were comparable to rates reported elsewhere in the country [26]. In addition, limiting the analysis of performance to a reference standard of fourth-generation EIA and Western blot (equivalent to third-generation HIV testing) did not change the performance of the rapid testing. There may have been undocumented user errors during home-based testing with RDT despite the implementation of a quality assurance programme including a proficiency testing. Another factor contributing to the reduced sensitivity observed could have been the selection and sequence of RDT kits used in the survey's serial testing algorithm. The algorithm had Determine as a screening test and UniGold as confirmatory. Studies of laboratory-based comparisons of RDT test kit performance have demonstrated lower than expected sensitivities with both Determine and UniGold test kits in certain settings. Gawalingo et al. reported a sensitivity of 97.3% for a serial algorithm which used Determine as the screening test [27]. Kosack et al. demonstrated a sensitivity of 96.2% for UniGold in a head-to-head comparison of eight RDT kits in the laboratory [28]. Because survey specimens were not tested by any other RDT in the laboratory, the contribution of RDT selection to the reduced sensitivity observed cannot be ruled out. In our study, there may have additional population-level factors such as gender and geographical location as well as others yet to be determined which may have affected RDT performance, as also reported by Kosack et al. [28]. Lastly, individuals who were false negative may

have been recently infected with detectable viral loads and higher CD4 cell counts. This is supported by the association between false-negative HIV results with positive LAg avidity assay which may indicate recent infection although false recency can occur [29]. However, the LAg assay is designed to detect infections up to 6 months in duration, a duration by which most HIV tests should be able to detect sufficient antibodies in the blood. Falsely negative individuals in the study could also have been on long-term ART. A few studies have documented low antibody titres and even seroreversion with long-term ART initiated during acute or early infection and continued long term with sustained viral suppression. Early ART initiation has also been associated with delayed antibody maturation following infection leading false recency of infection [30,31]. Retesting individuals on ART is currently not recommended [8].

Our analysis presents the performance of RDT during community-based household HIV testing. The analysis included a large group of randomly selected individuals giving relatively precise estimates of rapid HIV test performance and minimizing selection bias. Enrolling and testing one individual per household likely minimized clerical errors related to mixing-up participant results or specimens. In addition, there was laboratory confirmation of HIV status for all tested in the home, allowing direct comparison of RDT test performance. However, only participants who wanted home testing were tested, thus bringing bias in. For example, because of this self-selection, 14 participants who had self-reported being HIV positive including three reporting current or past ART use were also enrolled. However, a sensitivity analysis excluding these participants produced similar RDT performance.

Our study had a few limitations. Previous HIV testing and ART use were self-reported and not verified in the laboratory, so we were unable to determine the effect of retesting and ART use on test performance. Although a few individuals self-reported taking ART at enrolment ($n = 3$), it would have been ideal to validate this by testing plasma ARV levels in the laboratory. Although proficiency testing was conducted biweekly, there was incomplete and inconsistent documentation of user errors, storage and environmental conditions under which test kits were stored or used, all of which can affect RDT performance in the field. Lastly, the use of a less specific reference standard, two fourth-generation IAs, despite availability of more specific tests was another limitation of this analysis. This reference standard was used in order to mirror the reference standard used for resolving discrepant results in the national HIV testing programme. The use of fourth-generation testing with Western blot which showed comparable performance showed that this was not a major limitation. Despite these limitations, our study provides valuable information and lessons on the performance of RDT in home-based testing settings. Whether the lower than expected sensitivity observed in this study is due to the test or operator performance, the need to strengthen systems for correct storage of test kits and quality assurance programmes and using the results thereof to improve quality cannot be understated.

In conclusion, our study showed high accuracy using the RDT algorithm and the potential for the large-scale roll-out of community-based testing. However, reduced sensitivity with higher than expected false negatives associated with recent infection was observed. As the RDT algorithm showed high accuracy and ability to reliably identify the majority of HIV infections, its use in community-based HIV testing programmes should be promoted and scaled up as it reaches more people. However, messaging on the potential for false positives and false negatives should be included in HIV testing programmes and nucleic acid amplification testing considered for those on PrEP. In addition, the national HIV testing programme should regularly monitor and validate the rapid HIV testing algorithms and revised these as guided by the findings.

Authors' affiliations

¹Centre for HIV and STIs, National Institutes of Communicable Diseases, Sandringham, South Africa; ²School of Public Health, University of the Witwatersrand, Johannesburg, South Africa; ³Centre for the AIDS Programme of Research in South Africa (CAPRISA), Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa; ⁴Research Department, Epicentre AIDS Risk Management (Pty) Limited, Paarl, Cape Town, South Africa; ⁵Centres for Disease Control and Prevention (CDC), Pretoria, South Africa; ⁶Division of Virology, School of Pathology, University of the Witwatersrand, Johannesburg, South Africa

Competing interests

The authors have no competing interest to declare.

Authors' contribution

AP, ABMK, CC, DK and MG conceived the study; CC and DK were responsible for data collection and quality assurance; TK, AG and LL analysed the data, TK drafted the manuscript. All authors have read and approved the final version of the manuscript.

Disclaimer

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Funding

This study was funded by the US President's Emergency Plan for AIDS Relief (PEPFAR) through the US CDC under terms of the co-operative agreement 3U2GGH000372-02 W1. ABMK is supported by a joint South African Medical Research Council – National Institutes of Health grant [R01HD083343]. The content is solely the responsibility of the authors and does not necessarily represent the official views of any of the funders.

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Short report

False-negative HIV tests using oral fluid tests in children taking antiretroviral therapy from Harare, Zimbabwe

Ioana D. Olaru¹, Grace McHugh², Suba Dakshina², Edith Majonga^{2,3}, Ethel Dauya², Tsitsi Bandason², Katharina Kranzer³, Hilda Mujuru⁴ and Rashida A. Ferrand^{2,3§}

[§]**Corresponding author:** Rashida A. Ferrand, Clinical Research Department, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK. (rashida.ferrand@lshtm.ac.uk)

Abstract

Introduction: Rapid diagnostic tests (RDT) for HIV infection have high sensitivity and specificity, but in the setting of longstanding antiretroviral therapy (ART), can give false results that can lead to misinterpretation, confusion and inadequate management. The objective of this study was to evaluate the proportion of falsely negative results of a RDT performed on oral fluid in HIV-infected children on longstanding ART.

Methods: One hundred and twenty-nine children with known HIV infection and receiving ART were recruited from the HIV Clinic at the Harare Central Hospital, Zimbabwe. HIV testing was performed on oral fluid and on finger-stick blood.

Results and Discussion: Children included in the study had a median age of 12 years (IQR 10–14) and 67 (51.9%) were female. Median age at HIV diagnosis was 5 years (IQR 3–6) and the median time on ART was 6.3 years (IQR 4.3–8.1). The oral fluid test was negative in 11 (8.5%) patients and indeterminate in 2 (1.6%). Finger-stick blood test was negative in 1 patient. Patients with a negative oral fluid test had a higher CD4 cell count (967 vs. 723 cells/mm³, $p = 0.016$) and a longer time on ART (8.5 vs. 6 years, $p = 0.016$).

Conclusions: This study found that a substantial proportion of false-negative HIV test results in children on longstanding ART when using an oral fluid test. This could lead to misinterpretation of HIV test results and in the false perception of cure or delayed diagnosis.

Keywords: HIV; misdiagnosis; rapid diagnostic test; oral fluid test; children

Received 15 December 2016; Accepted 8 June 2017; Published 29 August 2017

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Introduction

Rapid diagnostic tests (RDT) for HIV infection using whole-blood specimens have been used globally since 2005 [1]. These RDTs have high sensitivity, are easy to perform, require little or no infrastructure, and have a relatively low cost and a rapid turn-around time making them optimal for low-resource, high HIV burden settings. However, as with any test, the performance of the test will depend on its inherent sensitivity and specificity and the prevalence of the condition being tested for. The problem of false-positive test results especially in the context of low HIV prevalence is well recognized. Serial testing with a highly sensitive test followed by a confirmatory test with high specificity addresses this issue [2].

Although RDTs have been widely used both in health facilities and in community-based HIV testing and counselling approaches, a key barrier remains the reliance of a client making contact with a provider and receiving the test result from the provider, who may be known to the client. In recent years, there has been increasing interest in promoting self-testing as a strategy to address these

barriers. Self-testing would enable individuals to undergo HIV testing confidentially and without concern about unwanted disclosure of their status to others. A recent meta-analysis of studies including adults at risk for HIV infection showed that HIV RDTs performed on blood had sensitivities and specificities exceeding 98–99% [3]. Oral fluid tests (OFTs) are RDTs that detect salivary HIV antibodies, and have been shown to have comparable performance to blood-based RDTs. As with blood-based RDTs, a positive OFT result can be confirmed by a subsequent blood-based test. In 2012, the first OFT received approval by the Food and Drug Administration as a home-use HIV kit for self-testing. The use of an OFT as a self-testing strategy has been demonstrated to be highly acceptable and accurate in Africa [4,5].

OFTs are particularly attractive for use in children because of their non-invasiveness. Studies have demonstrated a slow but persistent loss of HIV-specific antibodies in highly suppressed HIV-infected children and adolescents that may lead to false-negative results in blood-based RDTs [6]. HIV antibody titres in saliva are lower than antibody titres in blood,

which may make OFTs more prone to false-negative results [3]. This appears to be more frequently encountered in the setting of longstanding ART and in individuals receiving pre-exposure prophylaxis (PrEP) [7,8]. We recently observed several cases of false negative HIV tests using OFT among children and adolescents taking antiretroviral therapy. Although this has already been described to occur in adults, there are no studies focusing on the paediatric population [9]. To further investigate this, we systematically evaluated the performance of the OFT compared to the blood-based RDT among perinatally HIV-infected children aged 7–18 years established on ART.

Methods

The study was conducted in 2016 and was nested within an ongoing clinical cohort study among perinatally HIV-infected children on ART. Children with HIV who had been receiving ART for at least 18 months were recruited from the HIV Clinic at the Harare Central Hospital, Zimbabwe. HIV testing was performed using Ora-Quick ADVANCE HIV 1/II™ OFT (OraSure Technologies, Bethlehem, USA) for oral fluid and concurrently using a finger-prick whole-blood sample (Alere Determine HIV 1/2, Alere Technologies, Jena, Germany). Testing was performed as per the instructions of the manufacturer by trained nurses. The nurse who performed the test was blinded to the result of the other test. CD4 count was assessed using the Alere PIMA CD4 analyser, and viral load was measured using GeneXpert HIV-1 Viral Load (Cepheid, Sunnyvale, CA). Demographic details, age at ART initiation and duration of ART use were collected.

Statistical analysis was performed using STATA version 14 (Stata-Corp, TX, USA). The Mann–Whitney U-test and Student’s t-test were used to evaluate for differences between groups for continuous variables. For categorical variables, the χ^2 test was used. Multivariable logistic regression was used to examine for factors associated with a false negative OFT. The level of significance was set at $\alpha = 0.05$.

Ethical approval for the parent study was obtained from the Medical Research Council of Zimbabwe, the Biomedical Research and Training Institute Institutional Review Board

and the London School of Hygiene and Tropical Medicine Ethics Committee. Written informed consent from guardians and assent from participants were obtained. Specific verbal consent was also obtained to perform OFTs and finger-prick samples.

Results and discussion

In total 129 participants were enrolled, with median age 12 years (IQR 10–14), and 67 (51.9%) being female (Table 1). The study participants had been diagnosed with HIV infection at a median age of 5 years (IQR 3–6) and the median duration on ART was 6.3 years (IQR 4.3–8.1). At the time of the OFT, the median CD4 cell count was 747 cells/mm³ (IQR 474–989) and 30 (34.9%) had a viral load exceeding 1000 copies/ml. The OFT was negative in 11 (8.5%) patients and indeterminate in two (1.6%). Finger-prick blood tests were negative in one patient (0.8%) who also had a negative OFT. Patients with a negative OFT had a higher CD4 cell count (967 vs. 723 cells/mm³, $p = 0.016$), a longer time on ART (8.5 vs. 6 years, $p = 0.018$) and were more likely to be girls (76.9% vs. 49.1%, $p = 0.057$). Furthermore, children with a negative OFT had a median age at ART initiation of 4.5 years, while those with a positive test had a median age of 6.2 years although this was not statistically significant ($p = 0.138$). Only 5 (3.9%) children were started on ART within their first year of life. There was no association between age at ART initiation and a false-negative OFT result. While this was a pre-defined variable to be included in multivariable analysis, this was not done due to a strong collinearity with duration of ART. Notably, 64% of those with a positive OFT had a viral load <1000copies/ml compared to 78% of those with a false-negative OFT result.

This study shows that a substantial proportion of children and adolescents receiving ART have a false-negative or indeterminate HIV test result using an OFT. Significantly more false-negative results occurred using an OFT compared to a whole-blood-based HIV RDT. While false-negative RDT results, can be due to technical issues such as inappropriate performance and self-interpretation of the test, this was not the case in this study where HIV testing was performed by

Table 1. Characteristics of patients by oral mucosal test result

| | Total <i>n</i> = 129 | Positive OMT <i>n</i> = 116 | Negative or indeterminate OMT <i>n</i> = 13 | <i>p</i> -Value |
|---|-------------------------|-----------------------------|---|-----------------|
| Female, <i>n</i> (%) | 67 (51.9) | 57 (49.1) | 10 (76.9) | 0.057 |
| Age at study visit (years), median (IQR) | 12 (10–14) | 12 (10–14) | 12 (11–15) | 0.464 |
| Age at HIV diagnosis (years), median (IQR) | 5 (3–6) | 5 (3–6) | 4 (1–7) | 0.415 |
| Age at ART initiation (years), median (IQR) | 6 (4–8.3) | 6.2 (4.1–8.4) | 4.5 (1.4–7) | 0.138 |
| Time on ART (years), median (IQR) | 6.3 (4.3–8.1) | 6 (4.1–8) | 8.5 (7.2–9.4) | 0.018 |
| Current CD4 cell count (cells/ μ l), median (IQR) | 747 (474–989) | 723 (457–928) | 967 (754–1414) | 0.016 |
| *Viral load <1000 copies/ml, <i>n</i> (%) | 56 (65.1) | 49 (63.6) | 7 (77.8) | 0.400 |

*data available for 86 participants only. Of the total number of individuals where the viral load measurement was available, 9 had a negative or indeterminate OMT and 77 had a positive OMT.

trained nurses certified to provide HIV testing, and the oral fluid and the blood-based RDTs were performed concurrently [10].

False-negative test results on blood-based antibody tests have been shown to occur very early or very late in the course of disease [11], as well as a slow loss of HIV-specific antibodies among children with longstanding ART [6]. In addition, false-negative HIV tests have been reported in infants started on ART therapy soon after birth who were HIV DNA PCR-positive [12,13]. This may be explained by the decreased antigen presentation due to longstanding suppressed viral replication. Similarly, it could also be associated with the time between infection and ART initiation. For example, false-negative tests have been reported in children with perinatal HIV infection who were started on ART within the first months of life [14]. Furthermore, PrEP was shown to be associated with a delayed time to development of a reactive OFT when compared to placebo [15]. Since there appears to be an association between the early initiation of ART and test performance, false-negative OFTs while on treatment may become more common in both paediatric and adult populations due to the global move towards immediate treatment initiation following a positive HIV test. This underscores the importance of patient counselling to understand the implications of HIV infection and therapeutic goals for ART.

The sensitivity of the oral fluid-RDTs is high, reaching up to 100% (95%CI 97.9–100) when used for HIV screening of individuals who have never received ART [16]. However, this does drop among those who are taking ART. A longer duration of ART use and a high CD4 cell count were independently associated with a false-negative OFT in our study (Table 2). Those with a suppressed viral load appeared more likely to have a false-negative test, although we were not able to formally test for this association due to the large proportion of participants on whom viral load data was unavailable. Taken together, these findings imply that in this age group, those who have been on longstanding ART and robust immunological status have too low levels of antibodies to be detectable by OFTs. Although not statistically significant, an interesting finding was the higher rates of false-negative OFT test in females, although there was no association of gender with false-negative tests.

Oral fluid-based RDTs are an attractive test for self-testing, as it is convenient to use and ensures anonymity and confidentiality [17]. In some settings, oral fluid-based tests are available over-the-counter or through online purchase. In addition, the World Health Organization is encouraging countries to initiate pilot projects to

implement and evaluate effective strategies for HIV self-testing as a means of achieving universal coverage of HIV testing [18]. Belief in faith healing or in the use of alternative treatments to cure HIV has been commonly reported in some populations with individuals living with HIV undergoing retesting to check for cure [19]. In a study in Tanzania, 44% of participants to a study believed that certain alternative treatments can cure HIV [20], and in another study seeking cure at a faith healer was associated with a significant decrease in treatment adherence [21]. In the absence of adequate counselling and patient education, a false-negative test result may lead to a wrong perception of cure, leading to ART interruption and exit from HIV care [12]. Additionally, with the scale up of PrEP, there is a possible risk of delayed HIV diagnosis given the longer time required for OFT to become positive in individuals taking PrEP. Furthermore, false-negative tests might cause the underestimation of HIV prevalence in surveys if participants under-report their HIV status. In a recent survey we conducted among 7–18 year olds, 12.9% of HIV-infected participants had a false-negative oral-fluid-based HIV test result (*manuscript in preparation*).

The limitation of this study is that it included a relatively small number of children from one centre and the lack of a longitudinal assessment. In addition, viral load tests were missing in a third of patients and therefore the association between viral load suppression and false-negative OFT test results could not be reliably examined.

In conclusion, 10% of older children and adolescents with HIV infection who were on longstanding ART had falsely negative or indeterminate HIV test results when using the oral fluid HIV test. Awareness of the possibility of false-negative results among healthcare providers and patients taking ART as well as among clients accessing PrEP is critical, as self-testing is scaled up. Clear counselling and appropriate messaging are important to avoid misinterpretation of HIV test results, which could result in the false perception of cure or delayed diagnosis of HIV infection among those accessing PrEP. Additionally, improving sensitivity of OFTs, counselling to prevent their use in individuals already diagnosed with HIV infection and new testing strategies are of paramount importance to avoid confusion and misunderstanding.

Authors' affiliations

¹Division of Clinical Infectious Diseases, Research Center Borstel, Borstel, Germany; ²Biomedical Research and Training Institute, Harare, Zimbabwe; ³Department of Clinical Research, London School of Hygiene and Tropical Medicine, London, UK; ⁴Department of Paediatrics, University of Zimbabwe, Harare, Zimbabwe

Competing interests

The authors have no conflicts of interests to declare.

Authors' contribution

RAF and KK conceived the study. GM, SD, EM, HM and ED collected data. IDO performed data analyses with input from TB, and wrote the first draft. All authors have read and approved the final version

Acknowledgements

The study was funded by the Wellcome Trust. The funder of the study had no role in study design, data collection, data analysis, data interpretation or

Table 2. Factors associated with false-negative or indeterminate oral fluid-based HIV test

| Variable | OR (95% CI) | aOR (95% CI) |
|-------------------------------|---------------------|--------------------|
| Female | 4.66 (0.96; 22.47) | 4.21 (0.81; 21.89) |
| Duration of ART (years) | 1.30 (1.03; 1.64) | 1.31 (1.01, 1.69) |
| CD4 count >750 cells/ μ l | 10.00 (1.24; 80.61) | 9.50 (1.13; 79.62) |

writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Funding

The study was funded by the Wellcome Trust [grant 095878/Z/11Z].

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Research article

Prevalence and outcomes of HIV-1 diagnostic challenges during universal birth testing – an urban South African observational cohort

Karl-Günter Technau¹, Ahmad Haeri Mazanderani^{2,3}, Louise Kuhn⁴, Lucia Hans⁵, Renate Strehlau¹, Elaine J. Abrams⁶, Martie Conradie¹, Ashraf Coovadia¹, Ndileka Mbetse¹, Pamela M Murnane^{1,4}, Faezah Patel¹, Stephanie Shiao^{1,4}, Caroline T. Tiemessen^{2,7} and Gayle G. Sherman^{1,2§}

§Corresponding author: Gayle G Sherman, National Institute for Communicable Diseases, 1 Modderfontein Road, Sandringham 2031, Johannesburg, South Africa. Tel: +27 83 639 0351. (gayles@nicd.ac.za)

Abstract

Introduction: HIV-1 polymerase chain reaction (PCR) testing at birth aims to facilitate earlier initiation of antiretroviral therapy (ART) for HIV-infected neonates. Data from two years of universal birth testing implementation in a high-burden South African urban setting are presented to demonstrate the prevalence and outcomes of diagnostic challenges in this context.

Methods: HIV-exposed neonates born at Rahima Moosa Mother and Child Hospital between 5 June 2014 and 31 August 2016 were routinely screened at birth for HIV-1 on whole blood samples using the COBAS® AmpliPrep/COBAS® TaqMan (CAP/CTM) HIV-1 Qualitative Test, version 2.0 (Roche Molecular Systems, Inc., Branchburg, NJ, USA). Virological results were interpreted according to standard operating procedures with the South African National Health Laboratory Service. All neonates with non-negative results were actively followed-up and categorized according to HIV infection status as positive, negative, uncertain and lost to follow-up (LTFU).

Results: 104 (1.8%) of 5743 HIV-exposed neonates received a non-negative birth PCR result, for which laboratory data were available for 102 (98%) cases – 78 (76%) tested positive and 24 (24%) indeterminate. HIV infection status was confirmed positive in 83 (81%) infants, negative in 8 (8%), uncertain in 5 (5%) and LTFU in 6 (6%) cases. The positive predictive value (excluding cases of uncertain diagnosis and inadequate testing) following a non-negative HIV-1 PCR screening test at birth was 0.91 (83/91; 95% confidence interval: 0.85–0.96). Neonates testing positive at birth had significantly higher viral load (VL) results than those testing indeterminate at birth of 4.5 and 3.0 log copies/ml ($p = 0.0007$), respectively. Similarly, mothers of neonates with positive as compared to indeterminate birth test results had higher VLs of 4.5 and 2.7 log copies/ml ($p = 0.0013$), respectively. Half of neonates with an indeterminate birth test were shown to be HIV-infected on subsequent confirmatory testing, with time to final diagnosis 30 days longer for these neonates ($p < 0.0001$).

Conclusion: Indeterminate HIV-1 PCR results accounted for a quarter of non-negative results at birth and were associated with a high risk of infection in comparison to the risk of *in utero* transmission. Indeterminate birth results with positive HIV PCR results on repeat testing were associated with later final diagnosis. The HIV-1 status remains uncertain in a minority of cases because of repeatedly indeterminate results, highlighting the need for more sensitive and specific virological tests.

Keywords: HIV-1 PCR; early infant diagnosis; birth testing; indeterminate

Received 17 December 2016; Accepted 8 June 2017; Published 29 August 2017

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Introduction

Routine HIV-1 polymerase chain reaction (PCR) testing at birth for all HIV-exposed neonates was introduced into the South African Consolidated Guidelines in June 2015 in order to enhance access to care and thereby reduce HIV-related morbidity and mortality [1,2]. Although targeted birth testing amongst high-risk HIV-exposed neonates had been part of the national testing guidelines since 2013 [3], implementation of the new guidelines has seen the volume of testing amongst neonates less than seven days of age increase by

more than 40 times, with approximately 20,000 birth tests performed each month [4]. Importantly, whereas the rapid scale-up of birth testing has been associated with earlier diagnosis of intrauterine-infected neonates, there has also been an increase in the number of diagnostic challenges arising during the neonatal period.

Early infant diagnosis (EID) and treatment of HIV-1 is important due to the rapid progression of disease and early HIV-related morbidity and mortality [5–7]. Accurate diagnosis is critical to ensure that infected infants start

antiretroviral therapy (ART) early and that uninfected infants are not unnecessarily exposed to life-long treatment. The recent case of the “Mississippi baby”, and other similar cases, further highlights the importance of very EID on account of the potential for functional cure and loss of detectability associated with very early ART initiation. These cases also highlight the possibility that diagnostic difficulties at birth may hold important information for this field [8–10].

Although PCR testing methods used for EID have reported sensitivities and specificities nearing 100% [11,12], important limitations exist. High coverage of maternal ART and early infant prophylaxis, as well as early initiation of treatment in infants prior to receiving confirmatory test results, are associated with high-level exposure to ART at the time of testing. This in turn has been associated with suppression of viraemia in infected infants and loss of detectability and uncertain results when using diagnostic assays [13–17]. Although the association between ART and indeterminate HIV-1 PCR results has been highlighted, and recommendations made regarding the management of such infants, further research is required to ensure timely definitive diagnosis and successful linkage to care [18,19]. Waning antibody levels and seroreversion following early ART initiation are additional phenomena that make later diagnostic confirmation difficult [20–22]. In contrast to the possibility of loss of detectability, there is also concern that on account of the dramatic reduction in mother-to-child transmission in South Africa there will necessarily be a drop in the positive predictive value of all infant diagnostic testing methodologies, thereby increasing the risk of treating uninfected infants [23–25].

Hence, research is urgently needed to inform evidence-based management of infants with uncertain and indeterminate HIV-1 results during early infancy. We describe the prevalence and outcomes of diagnostic challenges associated with HIV-1 PCR testing at birth within a single health facility in a high-burden setting over a two-year period.

Methods

Neonates born to HIV-infected mothers were enrolled at Rahima Moosa Mother and Child Hospital (RMMCH), a tertiary institution situated in Johannesburg, South Africa, with approximately 1000 deliveries per month and an antenatal HIV prevalence of 23% [26]. The cohort of all infants born between 5 June 2014 and 31 August 2016 who had an HIV-1 PCR test at birth were included in the analysis. During this period, all HIV-exposed neonates were routinely screened at birth for HIV-1 using ethylenediaminetetraacetic acid (EDTA) anti-coagulated whole blood samples obtained by phlebotomy in the hours following delivery and sent to a diagnostic laboratory for testing. Those with a non-negative result were actively traced and followed up either at RMMCH or referred to local facilities if unable to return. Initially all neonates with a non-negative laboratory HIV-1 PCR result were initiated on combination ART and samples taken simultaneously for confirmatory testing on follow-up. This practice was based on findings (from the previous version of the current assay)

that neonates with an HIV-1 PCR result at birth, whether positive or indeterminate, were invariably found to have a confirmed HIV-1-positive infection status [27]. However, after it was found that some neonates who tested indeterminate at birth had negative confirmatory testing, this practice was stopped and only neonates with a clearly positive virological result were initiated on ART prior to awaiting confirmatory results. For those neonates following up at RMMCH, confirmatory testing was performed using the same qualitative HIV-1 PCR assay and/or a viral load (VL) test on EDTA anti-coagulated whole blood and plasma, respectively. Neonates with indeterminate or discordant results were retested at each clinic visit until a definitive HIV-1 status was determined. Qualitative PCR testing outside of RMMCH was performed on either EDTA anti-coagulated whole blood or whole blood dried blood spot (DBS) samples, depending on access to phlebotomy services. All mothers of neonates who underwent birth testing and received a negative result were recommended to follow up for additional testing to detect possible intra-partum and post-partum infection according to national guidelines [1].

Laboratory methods

Qualitative HIV-1 PCR and VL testing were performed at accredited (ISO 15189:2012) diagnostic laboratories using the qualitative and quantitative versions of the COBAS® AmpliPrep/COBAS® TaqMan (CAP/CTM) HIV-1 Test, version 2.0 (Roche Molecular Systems, Inc., Branchburg, NJ, USA). The CAP/CTM is a total nucleic acid real-time reverse transcriptase PCR assay that detects HIV-1 proviral DNA and RNA on whole blood, and HIV-1 RNA only on plasma, with a limit of detection of approximately 300 RNA copies/ml and limit of quantification of 20 RNA copies/ml, respectively [11,28]. All non-negative virological results were interpreted according to standard criteria used within the National Health Laboratory Service (NHLS) to distinguish clearly positive from indeterminate results. All qualitative HIV-1 PCR results with a cycle-threshold value of ≤ 33 and a relative fluorescence intensity ≥ 5 , and VL results with a quantified or higher than the linear range result (>7 log RNA copies/ml) were defined as clearly positive. Hence, all non-negative virological results with a cycle-threshold of >33 and/or relative fluorescence intensity of <5 and VL results where RNA was detected but below the linear range of the assay (i.e. <1.3 log RNA copies/ml or <2.0 log RNA copies/ml for those samples that required a 1:5 dilution due to inadequate volume) were interpreted as indeterminate [29,30]. As a means for controlling for sample swap, genetic profiling of short tandem repeat loci was performed using the PowerPlex® 16 HS System (Promega Corporation, Madison, WI, USA) on the birth and subsequent samples of a patient who had a clearly positive virological result at birth followed by negative results on subsequent clinic visits.

Classifying HIV-1 status

The final diagnostic status of infants who received a non-negative HIV-1 PCR result was classified as follows:

- Positive HIV-1 infection status was based on two clearly positive virological results from samples taken at two different time points.
- Negative HIV-1 infection status was defined as an isolated indeterminate result followed by at least two negative confirmatory virological results taken at two different time points whilst not on combination ART.
- Uncertain HIV-1 infection status was defined as neonates with non-negative virological results that did not meet criteria for confirmed positive or negative infection status after repeat testing.
- Lost to follow-up (LTFU) was defined as patients who did not have sufficient follow-up testing to meet any of the above criteria.

We examined whether maternal factors, including VL, CD4 cell count or duration of ART pre-delivery, or infant factors, including age at screening test, age at diagnostic confirmation, VL and relation to commencement of daily dose nevirapine (ddNVP), were related to the screening or confirmatory testing outcomes in any way. All mothers identified as HIV-infected who delivered at RMMCH between June 2014 and August 2016 were invited to sign a data sharing informed consent form, approved by the Human Research Ethics Committee of the University of the Witwatersrand (M130653, M140760, M140555 and M140639). Clinical and laboratory data, recorded on paper, were captured into a routine REDCap database [31]. Data were analysed using SAS (Version 9.4, SAS

Institute Inc., Cary, NC, USA), and descriptive methods were used to present frequencies of events, medians and interquartile ranges (IQRs), the Cochran–Armitage Trend test to assess trends of indeterminate result outcomes and Kaplan–Meier method to assess time to diagnosis. We describe the events along the diagnostic process. Cases with complex or uncertain diagnostic events had file reviews and are presented as brief case reports.

Results

A total of 5743 (91%) of the 6309 HIV-exposed neonates born at the hospital were enrolled in the study of which 104 (1.8%) received a non-negative HIV-1 PCR result at birth. Of 102 (98%) neonates with laboratory data available, 78 (76%) were classified as positive and 24 (24%) were indeterminate according to laboratory criteria. After confirmatory testing, 83 (81%) infants were confirmed HIV-1 infected, amounting to an intrauterine transmission rate of approximately 1.4%, and 8 (8%) infants were assigned a negative HIV-1 infection status. The HIV-1 status of an additional 5 (5%) infants remains uncertain and 6 (6%) were LTFU. The positive predictive value (excluding cases of uncertain diagnosis and inadequate testing) following a non-negative HIV-1 PCR screening test at birth (i.e. all detected virological results) was 0.91 (83/91; 95% confidence interval: 0.85–0.96), and this increased to 1.0 when using NHLS cutoff values to distinguish positive from indeterminate results.

Amongst the 83 infants who were confirmed HIV-1 infected, 74 had a positive and 9 had an indeterminate HIV-1 PCR result at birth (Table 1). Of the 74 neonates

Table 1. Steps in establishing final HIV-1 infection status of 102 infants with non-negative birth PCR results

| First PCR result | | Second PCR result | | | Earliest VL result | | | Final HIV-1 infection status, [†] N (%) | | | |
|---|----|--|----|-------------------------|--------------------|--------------------------------------|-------------------------|--|------------------|----------|-------|
| Birth HIV-1 PCR test | n | Result | n | Age (days) [‡] | n | VL (log RNA copies/ml) [‡] | Age (days) [‡] | Positive | Uncertain | Negative | LTFU |
| Positive | 78 | Positive | 68 | 2 (1–9) | 66 | 4.48 (3.4–5.4) | 2 (1–8) | 68 | | | |
| | | Indeterminate | 4 | 4, 4, 8, 40 | 4 | <1.3 ^e , 2.58, 4.09, 4.56 | 68, 8, 4, 4 | 3 | 1 ^e | | |
| | | Not tested | 6 | | 3 | 4.30, 5.04, 6.62 | 1, 4, 94 | 3 | | | 3 |
| | | Total birth HIV-1 PCR positive results | | | | | | | 74 (95) | 1 (1) | |
| Indeterminate | 24 | Positive | 5 | 6 (6–12) | 4 | 2.29, 2.96, 3.05, 4.45 | 12, 0, 6, 1 | 5 | | | |
| | | Indeterminate | 7 | 8 (6–24) | 6 | TND ⁱ , 3.1 (3.0–3.2) | 2 (1–8) | 4 ^{a–d} | 2 ^{g,i} | | 1 |
| | | Negative | 11 | 7 (3–11) | 10 | TND (n = 9), 2.82 ^f | 8 (4–10) | | 2 ^{f,h} | 8 | 1 |
| | | Not tested | 1 | | 0 | | | | | | 1 |
| Total birth HIV-1 PCR indeterminate results | | | | | | | 9 (38) | 4 (17) | 8 (33) | 3 (13) | |
| Total, n = 102 | | | | | | | | 83 (81) | 5 (5) | 8 (8) | 6 (6) |

[†]Positive HIV-1 infection status was defined as two positive virological results from samples taken at two different time points. Negative HIV-1 infection status was defined as an isolated indeterminate result followed by at least two negative virological results taken at two different time points whilst not on combination ART. Uncertain HIV infection status was defined as initial non-negative virological results that did not meet either of the above criteria. Lost to follow-up (LTFU) was defined as insufficient follow-up testing to meet the above criteria.

[‡]Individual results are displayed for ≤4 cases and median (interquartile range (IQR)) for ≥5 cases.

PCR: polymerase chain reaction; VL: viral load; TND: target not detected.

The superscripts ^{a–d} in column Positive and ^{e–i} in column Uncertain indicate the cases presented in more detail in Figure 1(a,b), respectively.

who screened positive, 72 had a retrievable confirmatory VL result, with a median value of 4.5 log copies/ml (IQR: 3.4–5.4), which was significantly higher than the 9 neonates with indeterminate results at birth who had a median VL of 3.0 log copies/ml (IQR: 2.8–3.2, $p = 0.0007$). All eight neonates who were diagnosed as uninfected had an isolated indeterminate screening result with at least two subsequent undetected virological results. Three of these infants repeatedly tested negative following all ART exposure cessation (including ddNVP prophylaxis and potential ingestion in breast milk of maternal ART) and five tested negative whilst still on ddNVP prophylaxis. Excluding the six infants who were LTFU, infection status could be clearly confirmed in the majority of cases (87 (91%) of 96 cases) by repeat virological testing on follow-up. However, in 9 (9%) of 96 cases a clear diagnosis could not be made on immediate follow-up on account of testing yielding repeatedly indeterminate virological results or negative results within the context of combination ART pressure. In four of the

nine cases (cases a–d, [Figure 1\(a\)](#)), ongoing testing eventually confirmed a positive HIV-1 infection status while in five cases (cases e–i, [Figure 1\(b\)](#)) the diagnosis remains uncertain despite further testing.

Samples for HIV-1 PCR screening at birth were taken at a median age of 14 h (IQR: 7–23) and were not significantly different ($p = 0.52$) between neonates with a positive birth result as compared to neonates with an indeterminate result ([Table 2](#)). However, time to diagnosis was significantly later for infants with an indeterminate screening result ($p < 0.0001$), which was confirmed as either infected or uninfected on a sample taken at a median age of 32 days (IQR: 14–180) by Kaplan–Meier analysis ([Figure 2](#)). For infected infants the time to confirmation was also significantly longer ($p = 0.0004$) for infants who had indeterminate HIV-1 PCR tests at birth (31 days (IQR:14–43)) compared to infants with a positive birth test (2 days (IQR: 1–8)). Duration of maternal antenatal ART exposure was not significantly different

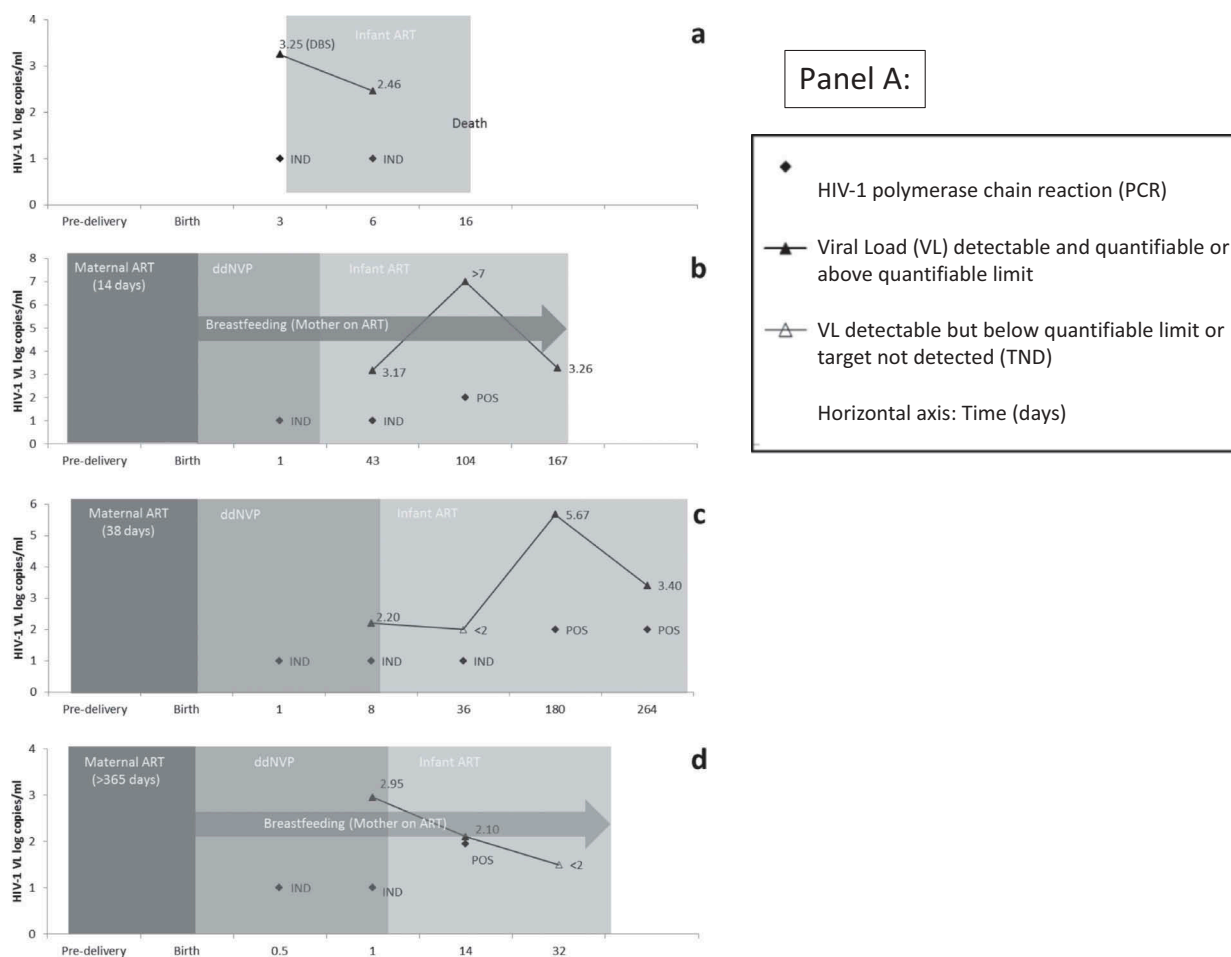


Figure 1. (A) and (B): HIV-1 PCR and viral load (VL) results in cases with positive (a–d) and uncertain HIV infection status (e–i), respectively. The time periods for which maternal antiretroviral therapy (ART), infant prophylaxis of daily dose nevirapine (ddNVP) and infant ART were given are represented by progressively lighter shades of grey. HIV-1 PCR tests were all done on whole blood and VL tests performed on plasma except where DBS is indicated. Due to space constraints some later repeat PCR negative or VL TND results were omitted (cases f–h).

DBS: dried blood spot; POS: positive; IND: indeterminate; NEG: negative; ART: antiretroviral therapy.

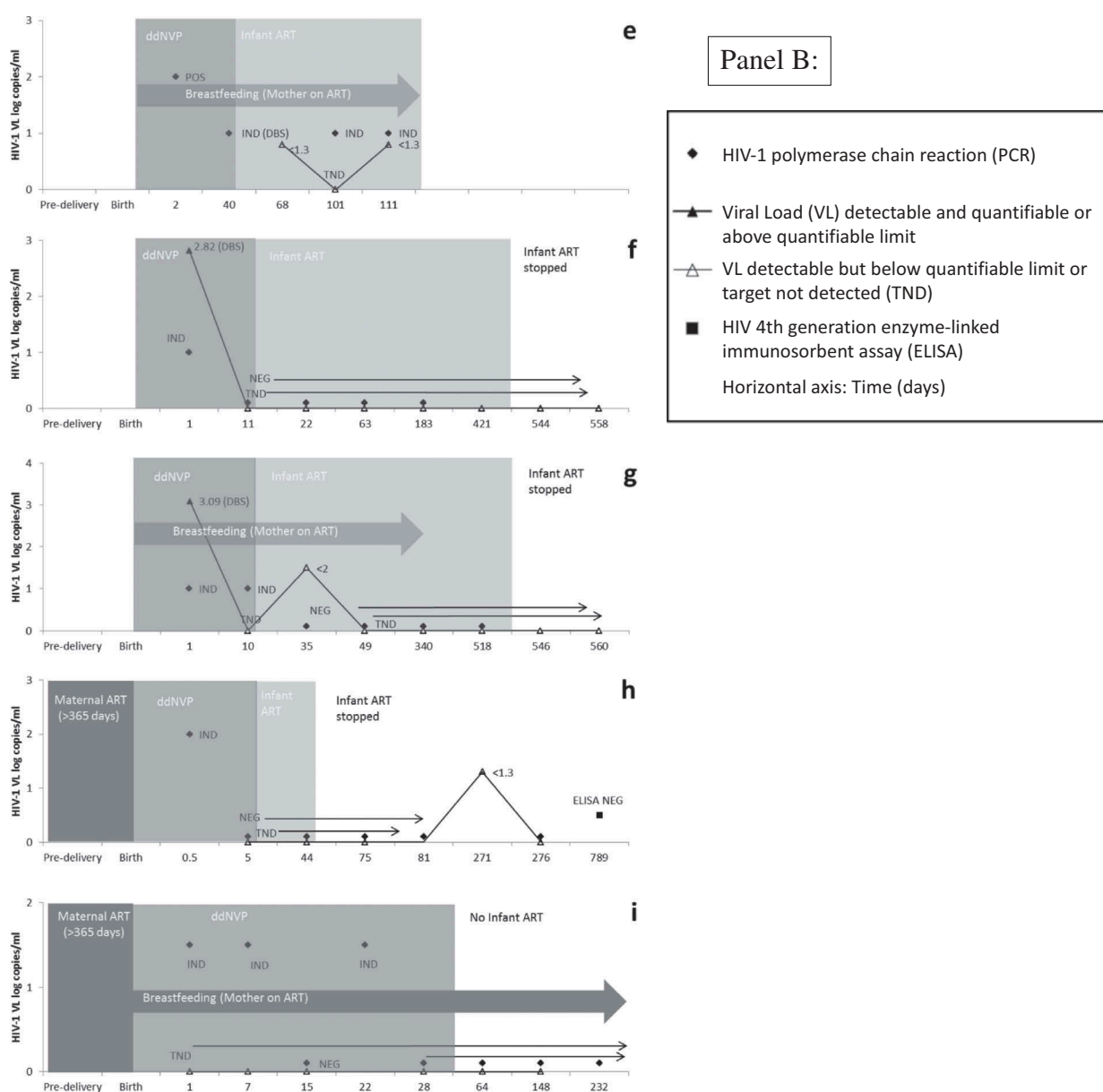


Figure 1. (continued).

between infants with a positive birth test as compared to infants with an indeterminate birth test, but maternal VL values were significantly different ($p = 0.0013$) with mothers of neonates with screening indeterminate results having a lower median baseline VL result (2.7 vs. 4.5 log copies/ml). There was a trend towards a higher CD4 count in mothers of neonates who tested indeterminate at birth but this was not statistically significant ($p = 0.059$). The probability of being confirmed as infected was 99% for infants with an initial screening positive result (one case remains with an uncertain diagnosis) versus 43% for infants with a screening indeterminate result ($p < 0.0001$). When stratifying neonates by

final diagnostic status (positive, negative or uncertain), there was a significant difference only in maternal VL ($p = 0.0008$) when comparing those with a confirmed positive HIV-1 status ($n = 68$) median 4.5 (IQR: 3.4–5.1), uncertain HIV-1 status ($n = 4$) median 2.8 (IQR: 2.4–3.4) and confirmed negative HIV-1 status ($n = 7$) median 1.9 copies/ml (target not detected–3.9). Four (17%) of 24 neonates who tested indeterminate and 7 (9%) of 78 neonates who tested positive at birth ($p = 0.28$) died during the course of this study.

Cases a–d (Figure 1(a)) are all examples of confirmed infected infants where the diagnosis took longer to make on account of confirmatory indeterminate test results that were

Table 2. Associations between screening birth HIV-1 PCR results and maternal and infant factors

| | HIV-1 PCR positive | HIV-1 PCR indeterminate | <i>p</i> |
|--|--------------------|-------------------------|----------|
| <i>N</i> | 78 | 24 | |
| No maternal ART exposure, <i>n</i> (column %) | 25 (32) | 5 (21) | 0.29 |
| Maternal ART exposure pre-delivery, <i>n</i> (%) | 53 (68) | 19 (79) | |
| 0–12 weeks | 21 (40) | 6 (32) | 0.48 |
| 12–26 weeks | 20 (38) | 6 (32) | |
| >26 weeks | 12 (23) | 7 (37) | |
| Median (IQR) weeks ART exposure | 16 (7–23) | 18 (3–135) | 0.61 |
| Maternal viral load (VL) data available, <i>n</i> (%) ^a | 61 (78) | 19 (79) | 0.92 |
| Median (IQR) log copies/ml | 4.5 (3.7–5.0) | 2.7 (1.9–4.3) | 0.0013 |
| Maternal CD4 cell count data available, <i>n</i> (%) | 75 (96) | 22 (92) | 0.37 |
| Median (IQR) maternal CD4 cell count (cells/μl) ^a | 280 (168–472) | 406 (264–608) | 0.059 |
| Median (IQR) age (hours) when birth sample taken | 13.7 (8.6–19.7) | 10.4 (5.3–20.9) | 0.52 |
| Nevirapine timing data available, <i>n</i> (%) | 59 (76%) | 18 (75%) | 0.84 |
| Blood for PCR was collected before NVP, <i>n</i> (%) | 4 (7) | 3 (17) | 0.34 |
| Median age (days) at final confirmation of HIV status (IQR) | 2 (1–8) | 32 (14–180) | <0.0001 |
| Lost to follow-up, <i>n</i> (%) | 3 (4) | 3 (13) | 0.14 |
| Final status, <i>n</i> (%) | | | |
| Confirmed infected | 74 (99) | 9 (43) | <0.0001 |
| Confirmed uninfected | 0 | 8 (38) | |
| Uncertain | 1 (1) | 4 (19) | |

^aMedian time of maternal VL (0.2 weeks after delivery (IQR: 0–2)) and CD4 (5 weeks before delivery (IQR: 15 weeks before–0.2 weeks after)) blood draws relative to delivery were not significantly different between the groups for each test. PCR: polymerase chain reaction; ART: antiretroviral therapy; IQR: inter-quartile range.

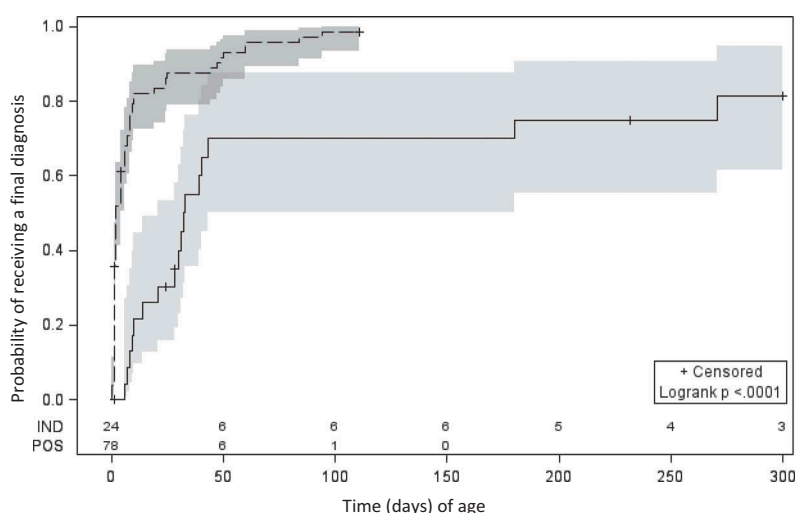


Figure 2. Kaplan–Meier curves of time to successful diagnosis (infected or uninfected) comparing infants with screening birth PCR positive (*n* = 78) to indeterminate (*n* = 24) results.

POS: positive (dashed line), IND: indeterminate (solid line) HIV-1 polymerase chain reaction (PCR) screening result with shaded areas representing 95% confidence intervals and numbers at risk above the x-axis. Censoring occurred at last test where diagnosis remained uncertain or at last visit date where loss to follow-up occurred.

unable to provide an immediate definitive diagnosis. In all four cases, infants repeatedly tested HIV-1 PCR indeterminate associated with low-grade viraemia (around 2–3 log copies/ml).

The HIV-1 status of cases e–i remains uncertain (Figure 1(b)) because virological detection was noted in at least two separate samples, by virtue of a combination of positive or indeterminate HIV-1 PCR results and low but quantifiable or indeterminate VL results. However, the virological test results have not fulfilled the criteria for a positive HIV-1 infection status. The exception is case f who demonstrated virological detection on a single sample at birth that tested HIV-1 PCR indeterminate with a VL of 2.82 copies/ml on DBS. On account of the uncertain diagnosis associated with a single quantifiable virological result in this case, genetic profiling was performed which confirmed that the birth sample belonged to the correct patient and ruled out the possibility of a sample swap or contamination with another patient's sample. Cases f and g are similar in that both had an HIV-1 PCR indeterminate result at birth that was associated with a quantifiable VL, using leftover whole blood stored on a DBS card from the birth sample. Both cases were not exposed to maternal ART during the antenatal period. In case g the indeterminate result at birth was followed by an indeterminate result at 10 days of age (during ddNVP exposure) and an indeterminate VL at 35 days of age that was detectable but not quantifiable (<2 log copies/ml). All subsequent virological testing was negative. In both cases f and g, ART was stopped at 76 weeks of age and both have since had at least two undetected virological test results at ± 4 weeks off treatment. In case h, a birth indeterminate result was followed 270 days later by a single indeterminate VL and this case is being monitored closely. Case e, who was not followed up on site, had three indeterminate results after the birth PCR positive result, the first of which was tested on a DBS card and occurred during ddNVP exposure while the latter two occurred during combination ART exposure. To date, the patient has never had a quantified VL result on ART. Whereas case i is the only case with an uncertain diagnosis who was not initiated on combination ART, treatment was subsequently stopped for cases f–h on account of inadequate evidence to confirm a positive HIV-1 infection status. None of these cases had shown any evidence of rebound.

Discussion

Whereas HIV-1 status could be confirmed in the majority of neonates who received a non-negative HIV-1 PCR result, there were significant delays and challenges associated with infants who tested indeterminate at birth, comprising 24% of all non-negative screening tests. This group of neonates were found to carry a significant risk of having a positive HIV-1 infection status, confirmed in 43% of cases, and required extensive follow-up beyond a standard once off confirmatory test. Furthermore, four neonates who tested indeterminate at birth remain with an uncertain

diagnosis. Overall, 25–30% of infants with a non-negative result required a diagnostic phase of management that extended beyond a single repeat test.

Considering the high morbidity and mortality associated with HIV-1 infection amongst infants, and considering 4 out of 24 (17%) infants who tested indeterminate at birth died during the course of this study, the need for a rapid definitive diagnosis is clearly of paramount importance. However, with similar proportions of neonates who tested indeterminate at birth found to have a positive and negative HIV-1 status on subsequent testing, and initiation of treatment prior to confirmatory testing known to confound diagnosis, a balance needs to be struck between effectively managing suspected HIV-infection and unnecessarily committing a patient to ART. Although NHLS cutoff values used to distinguish positive from indeterminate results are associated with an improved positive predictive value of the assay, they are also necessarily associated with an increase in delayed diagnosis and uncertain HIV-1 status that requires close monitoring with repeated testing. Furthermore, indeterminate results pose difficulties not only for clinicians but also for primary caregivers and the family of infants given an uncertain HIV-1 diagnosis. Importantly, these caregivers may lose confidence in clinicians and the healthcare system in general if clinical staff are unable to provide a clear and timely diagnosis.

Although it remains to be determined whether indeterminate HIV-1 PCR results are more common at birth, indeterminate results are not a phenomenon associated solely with birth testing. Rather they occur within all age groups in which HIV-1 PCR testing is performed and have been described as a leading cause of non-negative results within South Africa's EID programme prior to the introduction of birth testing, comprising on average 16% of all non-negative results [18,32]. More data are needed to assess how birth testing affects the rates of indeterminate results. Whereas concerns surrounding sample swap and contamination are valid, and possibly account for some indeterminate results, they do not comprise the majority of such cases. Similarly, indeterminate results cannot simply be accounted for by citing a reduction in the positive predictive value of diagnostic assays within the context of declining mother-to-child transmission rates. Clearly, other factors are associated with uncertain and delayed diagnosis amongst HIV-infected neonates. Our study demonstrates a correlation between lower maternal and infant VL results in relation to indeterminate HIV-1 PCR results, suggesting that mechanisms of virological control, including ART and immunological factors, need to be considered when dealing with EID challenges. This further highlights the importance of time of testing, especially considering cases which tested positive at birth but received indeterminate confirmatory results during ART exposure. Sensitivity and specificity of HIV-1 PCR assays for EID were not measured in our study but sensitivity appears to be decreased by maternal and infant prevention of mother-to-child transmission prophylaxis [15–17], and the high proportion of indeterminate results in our study suggests a need for more sensitive and specific assays.

A total of 5 infants (5%), out of 102 neonates with a non-negative HIV-1 PCR result at birth, remain with an uncertain diagnosis. Three of these cases (f, g and h) tested indeterminate at birth and were started on ART on the day of confirmatory testing. This practice was based on findings from the previous version of the current assay that neonates with a non-negative HIV-1 PCR result at birth, whether positive or indeterminate, were invariably found to have a confirmed HIV-1 positive infection status [27]. However, once it was determined that this was not the case with the more sensitive CAP/CTM v2.0 assay, this practice was stopped. All three of these infants have since followed up on site, and treatment has been interrupted under close clinical supervision. The diagnosis of these infants remains uncertain as it has yet to be determined what the required length of time is for monitoring post-treatment cessation in order to exclude HIV-1 infection [24]. Case e represents the only infant with an uncertain HIV-1 status where combination ART has not been stopped and is also the only case, amongst those with an uncertain diagnosis, that tested HIV-1 PCR positive at birth. It is worth noting that this infant is being followed up outside of the study setting and that confirmatory testing was performed on a DBS sample only, without a simultaneous VL test, and that the volume of blood tested on a DBS sample (approximately 60 µl) is less than that used to test EDTA anti-coagulated whole blood samples (100 µl) and this may have had an impact on the confirmatory result.

As a collective, the diagnostic challenges described in this study raise important questions concerning EID, including the potential for antiretroviral prophylaxis to be associated with virological control and even “functional-cure”-type scenarios [9]. Furthermore, infants with multiple indeterminate virological results followed by loss of detectability raise fundamental questions regarding the mechanism of post-exposure prophylaxis and the possibility of transient or abortive infectious processes. Similarly, it remains to be determined whether isolated indeterminate results represent false detection or true infection associated with faster virological control.

Conclusions

Whereas the majority of neonates with a positive HIV-1 PCR test at birth were confirmed to be HIV-1 infected, indeterminate results were associated with uncertainty and diagnostic delay. Although indeterminate results comprised 24% of all non-negative birth tests in this study, true ongoing diagnostic dilemmas were rare with most cases resolving on repeat testing, and approximately half of these having a confirmed positive diagnosis and half confirmed negative. In some of these cases a quantifiable VL result confirmed the diagnosis whereas the repeat HIV-1 PCR test was indeterminate, suggesting a combination of virological testing methods may be beneficial when confirming HIV-1 infection. Essentially, the clinical requirements and social consequences of managing an infant with an indeterminate HIV-1 result make it critical that a timely and unequivocal diagnosis is established by the treating clinician and effectively communicated to the primary caregiver.

Authors' affiliations

¹Empilweni Services and Research Unit, Department of Paediatrics & Child Health, Rahima Moosa Mother and Child Hospital, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; ²Centre for HIV & STIs, National Institute for Communicable Diseases, Johannesburg, South Africa; ³Department of Medical Virology, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa; ⁴Gertrude H. Sergievsky Center, College of Physicians and Surgeons, and Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, USA; ⁵Department of Molecular Medicine and Haematology, Faculty of Health Sciences, University of the Witwatersrand, and National Health Laboratory Service, Johannesburg, South Africa; ⁶ICAP, Mailman School of Public Health, and Department of Pediatrics, College of Physicians & Surgeons, Columbia University, New York, USA; ⁷School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Competing interests

The authors have no conflicts of interest to disclose.

Authors' contribution

KT, LK, AC, GGS, RS, CTT, EJA: study design; KT, RS, FP, MC, NM, LH, AHM: data collection; KT, AHM, LK, GGS, RS, CTT, PMM, SS, EJA, FP, LH: data interpretation and analysis; KT, AHM, GGS, AC, LK, PMM, SS, LH, RS, FP, MC, NM, CTT, EJA: writing.

Acknowledgements

We gratefully acknowledge the hard work of Perry Hlalele for work in the laboratory as well as the clinicians at Rahima Moosa Mother and Child hospital for their contribution to the management of patients, particularly Drs Gill Sorour and Gary Reubenson. The study would not have been possible without the willing participants and tireless work of a dedicated study team. The study was supported in part by the National Institutes of Health U01 HD080441, PEPfAR/USAID and UNICEF. We thank the Data Safety and Monitoring Board (DSMB) of the associated LEOPARD study for reviewing some of these cases.

Funding

This work was supported in part by the National Institutes of Health U01 HD080441; PEPfAR/USAID; and UNICEF.

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Short report

Misdiagnosed HIV infection in pregnant women initiating universal ART in South Africa

Nei-yuan Hsiao^{1§}, Allison Zerbe², Tamsin K. Phillips³, Landon Myer³ and Elaine J. Abrams^{2,4}

[§]**Corresponding author:** Nei-yuan Hsiao, Division of Virology, Faculty of Health Sciences, University of Cape Town, Anzio Rd, Observatory, 7925, Cape Town, Cape Town 7925, South Africa. Tel: 27 21 4045200. (marvin.hsiao@uct.ac.za)
Clinical Trial Number: NCT01933477

Abstract

Introduction: Rapid diagnostic tests (RDTs) are the primary diagnostic tools for HIV used in resource-constrained settings. Without a proper confirmation algorithm, there is concern that false-positive (FP) RDTs could result in misdiagnosis of HIV infection and inappropriate antiretroviral treatment (ART) initiation, but programmatic data on FP are few.

Methods: We examined the accuracy of RDT diagnosis among HIV-infected pregnant women attending public sector antenatal services in Cape Town, South Africa. We describe the proportion of women found to have started on ART erroneously due to FP RDT results based on pre-ART viral load (VL) testing and enzyme-linked immunosorbent assay (ELISA).

Results: We analysed 952 consecutively enrolled pregnant women diagnosed as HIV infected based on two RDTs per local guideline and found 4.5% (43/952) of pre-ART VL results to be <50 copies/ml. After excluding 6 women who had detectable virus on subsequent VL measurements, ELISA was performed on the 37 remaining women. Of these, 3/952 (0.3%) HIV RDT diagnoses were found to be FP. We estimate that using ELISA to confirm all positive RDTs would cost \$1110 (uncertainty interval \$381–\$5382) to identify one patient erroneously initiated on ART, while it costs \$3912 for a lifetime of antiretrovirals with VL monitoring for one person.

Conclusions: Compared to the cost of confirming the RDT-based diagnoses, the cost of HIV misdiagnosis is high. While testing programmes based on RDT should strive for constant quality improvement, where resources permit, laboratory confirmation algorithms can play an important role in strengthening the quality of HIV diagnosis in the era of universal ART.

Keywords: HIV; diagnosis; rapid diagnostic test; specificity; ART; false positive; ELISA

To access the supplementary material to this article please see [Supplementary Files](#) under Article Tools online.

Received 16 December 2016 **Accepted** 15 May 2017 **Published** 29 August 2017

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Introduction

Rapid diagnostic tests (RDTs) detecting the HIV-1/2 antibodies are used globally to diagnose HIV infection. When performed optimally, RDTs are highly sensitive and specific. In a World Health Organization (WHO) report of HIV assays, laboratory studies evaluating eight RDTs observed a sensitivity of 99.4–100% and a specificity of 98.9–100% [1]. In addition to their comparable performance with the gold standard enzyme-linked immunosorbent assay (ELISA), RDTs are inexpensive, are easy to use and can be used at point-of-care. With recent evidence and recommendations favouring early antiretroviral treatment (ART) initiation [2–4] and the universal “test-and-start” approach [5], the use of RDTs to quickly diagnose HIV infection and facilitate immediate ART initiation will be critical in achieving the UNAIDS 90-90-90 goal in many low- and middle-income countries (LMIC).

However, the quality of RDT diagnostic services is highly dependent on user training and quality assurance of the performing facility. The HIV testing services (HTS) are often overburdened with the high service load and lack the

necessary training in quality assurance. As a result, the high sensitivity and specificity of RDT observed in assay evaluation studies may not translate to the same performance in real-world HTS. For example, according to a 2012 report, the level of testing process compliance among a sample of 38 South African health facilities was 3.4% with completion of registers, appropriate incubation time and post-test counselling cited as steps with the poorest compliance [6].

While poor RDT performance with high numbers of false-negative results in the field has been identified as a problem [7–9], false-positive (FP) RDT results are reported less frequently. Currently, South Africa follows the WHO-recommended strategy of using two RDTs to diagnose HIV infection in adults [10]. In the past, the majority of HIV-infected individuals in sub-Saharan Africa had started ART on the basis of two concordantly positive screening and confirmatory RDTs in the context of appropriate clinical or immunological criteria. With universal ART eligibility, there is concern that misdiagnosis of HIV infection and

inappropriate ART initiation could be more common because the safety net of the clinical and immunological screenings will be eliminated. While the WHO guidelines recommend retesting prior to ART initiation in order to minimize misclassification of HIV status, the retest policies are often centred on previously RDT-negative individuals. There is little guidance on how large national programmes might go about implementing this additional testing for individuals tested positive by RDTs. There are also few published analyses on the cost implication of such retesting. In South Africa where access to HIV viral load (VL) testing is good, pre-ART VL has been considered as one option for confirmation of HIV infection, but, again, there are few insights into the potential consequences of universal pre-ART VL testing.

In this study, we aim to estimate the proportion of FP RDT through laboratory confirmation using VL and HIV ELISA. We also compare the cost of different retesting strategies with potential inappropriate ART initiation in the test-and-start era.

Methods

Study population

This is a retrospective study examining the FP HIV misdiagnosis in a cohort of pregnant women attending antenatal services at a public sector primary care facility in Cape Town, South Africa, between 2013 and 2014. Following local algorithms based on the WHO-recommended two-test strategy, HIV diagnosis in this setting employs two third-generation HIV antibody RDTs: SD Bioline HIV-1/2 (Standard Diagnostics, Kyonggi-do, South Korea) used for screening and the Alere Determine HIV 1/2 (Alere, Waltham, MA, USA) used for confirmation [11].

As part of a larger study of ART in pregnancy [12], we conducted pre-ART VL testing (Abbott RealTime HIV-1) in consecutive HIV-infected pregnant women making their first antenatal clinic (ANC) visit who were not on ART or antiretroviral (ARV) prophylaxis according to self-report. Any pre-ART women who were aviraemic, defined as VL of <50 copies/ml, were further investigated. Some of the women included in this sample were diagnosed with HIV prior to ANC enrolment, while others were diagnosed during the current pregnancy.

Confirmation of HIV diagnosis

In order to detect the cases of FP HIV misdiagnosis in this population, we consider all women who reported RDT positive and were viraemic during the study as true HIV infection. Women who were identified as aviraemic per their pre-ART VL test and not found to have a subsequent viraemic episode were tested by a fourth-generation HIV ELISA (Enzygnost HIV Integrol4, Siemens, Marburg, Germany) which had a specificity of 99.9% and was optimized in the local laboratory for the purpose of confirmatory testing. Those who were both persistently aviraemic and found to be negative per confirmatory ELISA testing were considered to be HIV uninfected.

Cost of misdiagnosis

Based on current survival trends in adults in South Africa [13], we estimated that each misdiagnosis would be enrolled in the ARV programme for approximately 30 years. Using the rate of false positivity identified in this analysis, we estimated the costs to identify one erroneous ART initiation using a further RDT, confirmatory HIV ELISA testing, pre-ART VL followed by confirmatory ELISA among aviraemic individuals and confirmatory ELISA for those with CD4 >350 cells/mm³. All confirmatory test results are treated as 100% specific. The cost of laboratory testing is derived from the 2015 South African National Health Laboratory Service tariff, and the total programme cost of ART in sub-Saharan Africa is based on a previous published estimate [14]. Uncertainty intervals were calculated based on the 95% confidence interval (CI) of the FP point estimate. We also modelled the cost-comparing confirmatory algorithms above with the cost of treating a misdiagnosis across a spectrum of hypothetical RDT FP rate. Statistical analysis was performed on Stata 12 (Stata Corporation, College Station, USA).

Ethical approval

The study was reviewed and approved by the University of Cape Town Human Research Ethics Committee (HREC 451/2012) and the Columbia University Medical Center Institutional Review Board (IRB-AAAK8059). All women provided written informed consent prior to participation.

Results and discussion

This analysis included 952 consecutively enrolled pregnant women who were diagnosed with HIV based on RDT algorithms and who reported no current ART use. The demographic, clinical and laboratory parameters are shown in Table 1. At the time of pre-ART VL testing, the median gestational age for these women was 21 weeks (interquartile range [IQR] 15–27). For women who were diagnosed with HIV prior to the current pregnancy, the median time since HIV diagnosis was 43 months (IQR 21–70). The overall median CD4 cell count was 382 cells/mm³ (IQR 255–547), and the median VL among the viraemic women was 4.00 (IQR 3.47–4.58) log copies/ml.

In pre-ART VL testing, 43 women (4.5%) were aviraemic prior to ART initiation or ARV prophylaxis were investigated further as suspected FP from RDTs (Figure 1). Of these, 6 women had detectable virus on subsequent VL measurements; the remaining 37 underwent additional testing using ELISA. Three women were found to be HIV negative by ELISA, representing 7% of all aviraemic women (3/43) and 0.3% (3/952, 95% CI: 0.07–0.9) of all women previously identified as HIV infected by public sector HTS using RDT and who reported not being on ART at the time of entry into antenatal care. Background information on the three cases of misdiagnosis is provided in the supplementary information. The parameters used in the cost comparison and cost modelling are detailed in Table 2. Based on these findings we estimate that immediate use of an ELISA or a

Table 1. Study population characteristics.

| | Baseline VL ≥ 50 | Baseline VL < 50 | Total |
|---|-----------------------|--------------------|---------------|
| Number of women | 909 | 43 | 952 |
| Median age in years (IQR) | 27 (24–31) | 30 (25–33) | 27 (24–32) |
| Gestational age in weeks (IQR) | 21 (15–26) | 26 (19–32) | 21 (15–27) |
| Median time since diagnosis (months) (only among women previously diagnosed) | 43 (22–70) | 34 (15–69) | 43 (21–70) |
| New HIV diagnosis during current pregnancy (%) | 479 (53) | 25 (58) | 504 (53) |
| Median CD4 cells/mm ³ (IQR) | 373 (250–530) | 723 (510–919) | 382 (255–547) |
| Median VL copies/ml (IQR) | 10,109 (2956–38,175) | Not Applicable | |
| Median VL log copies/ml (IQR) | 4.00 (3.47–4.58) | Not Applicable | |

This table outlines the demographic, pregnancy, immunological and virological characteristics of pregnant women testing HIV positive by RDT. VL: viral load; IQR: interquartile range.

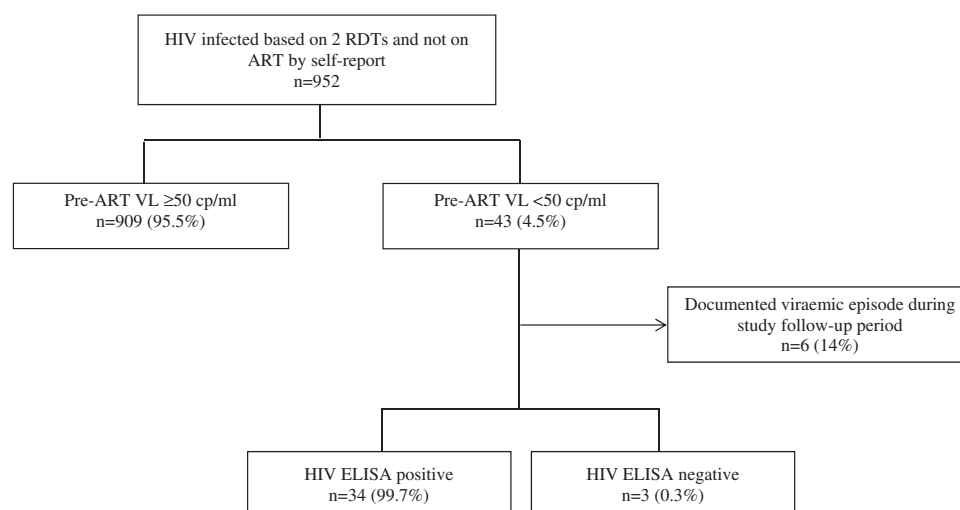


Figure 1. The study flow diagram. Our study examined 952 women entering antenatal care with HIV diagnosis based on two rapid diagnostic tests (RDTs) performed by the routine public sector HIV testing service. Viral load testing, including a pre-ART viral load (VL) test, was performed during their antenatal visits as part of clinical trial participation. Through HIV VL and HIV enzyme-linked immunosorbent assay (ELISA) testing in a subset of these women, we identified the proportion of false-positive RDTs.

third RDT alone as a confirmatory test to identify one patient erroneously initiated on ART would cost \$1110 (uncertainty interval, \$381–\$5382) or \$889 (\$305–\$4306), respectively. By limiting confirmatory testing to individuals with CD4 count >350 cells/mm³, the cost of ELISA would be further reduced to \$631 (\$217–3057) while still identifying all false positive results. By contrast, approximately \$6397 (\$2197–\$30,994) would be spent on confirmatory pre-ART VL testing, with further ELISA for aviraemic women, to identify a single patient erroneously initiated on ART. In comparison, based on the current estimate, the total programme cost of 30 years of ART would cost approximately \$13710 per misdiagnosis. Modelling the cost comparison across various hypothetical field FP rate suggest that while saving increases with higher FP rates, retest is cost saving even if the FP rate is as low as 0.1% (Figure 2).

Using a hypothetical RDT FP rate range of 0.05–15%, our model showed that the use of an additional RDT to confirm the initial RDT-based diagnosis will cost \$19–\$5600 to identify a single case of FP RDT. The cost of using a single ELISA to confirm RDT result ranges from \$23 to \$7000, while screening with VL followed by ELISA in an aviraemic individual costs between \$467 and \$140,000. The VL-ELISA algorithm is based on the study data that 4.5% of the pre-ART individuals are aviraemic. Confirming RDT-positive patients with CD4 >350 alone will cost \$13–\$3976 per positive patient identified. The cost of 30 years of ART and VL monitoring estimate is based on current Clinton Health Access Initiative (CHAI) reference and National Health Laboratory Service (NHLS) prices at \$110 per year of tenofovir, lamivudine and efavirenz fixed-dose combination therapy and \$20 VL testing per year.

Table 2. Parameters used for the cost-comparison analysis between the cost of living with the misdiagnosis in the South African ART programme and the cost of HIV status confirmation required to identify a single case of incorrect positive HIV diagnosis

| | Value | Source(s) |
|---|-------|---|
| Cohort characteristics | | |
| Proportion of pre-ART individuals with viral load <50 copies/ml | 0.045 | This study |
| Proportion of false-positive RDT | 0.003 | This study |
| Specificity of various methods of confirmation | 100% | Assumption |
| Proportion of pre-ART individuals with CD4 >350 | 0.56 | This study |
| Years living with misdiagnosis | 30 | Johnson et al. |
| Cost (USD) | | |
| Programme cost of early ART per person per year | \$457 | McGillen et al. |
| Third RDT | \$2.8 | South African National Health Laboratory Service tariff |
| Single ELISA | \$3.5 | South African National Health Laboratory Service tariff |
| Single viral load | \$20 | South African National Health Laboratory Service tariff |

ART: antiretroviral treatment; RDT: rapid diagnostic test; ELISA: enzyme-linked immunosorbent assay.

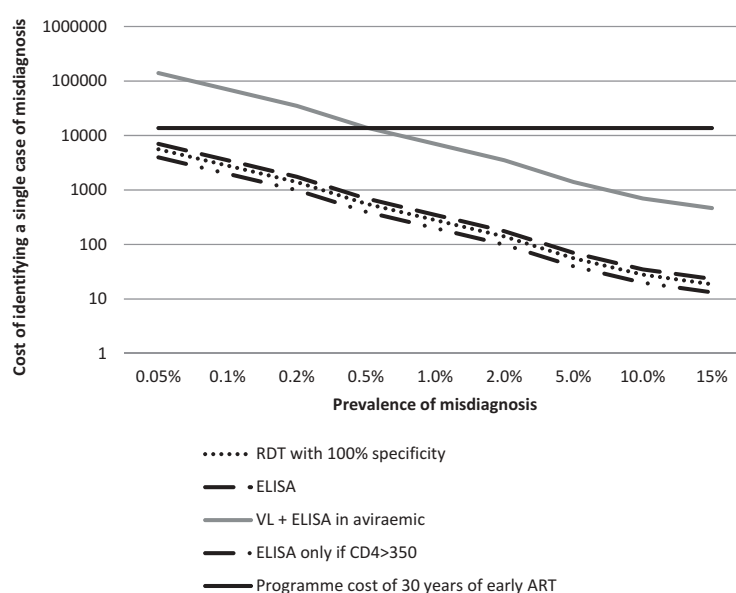


Figure 2. Estimated additional laboratory cost in order to identify a single case of false-positive (FP) rapid diagnostic test (RDT) by various testing algorithms.

Using a hypothetical RDT FP rate range of 0.05–15%, our model showed that the use of an additional RDT to confirm the initial RDT-based diagnosis will cost \$19–\$5600 to identify a single case of FP RDT. The cost of using a single ELISA to confirm RDT result ranges from \$23 to \$7000, while screening with VL followed by ELISA in an aviraemic individual costs between \$467 and \$140,000. The VL-ELISA algorithm is based on the study data that 4.5% of the pre-ART individuals are aviraemic. Confirming RDT-positive patients with CD4 >350 alone will cost \$13–\$3976 per positive patient identified. The cost of 30 years of ART and VL monitoring estimate is based on current Clinton Health Access Initiative (CHAI) reference and National Health Laboratory Service (NHLS) prices at \$110 per year of tenofovir, lamivudine and efavirenz fixed-dose combination therapy and \$20 VL testing per year.

Discussion

Our data in pregnant women attending antenatal services at a primary care facility in Cape Town, South Africa, highlight the importance of retesting when using RDTs as the sole diagnostic tool, particularly within the new test-and-start paradigm. Among this cohort of pregnant women who were not on ART or receiving ARV prophylaxis, we found that 0.3% of the HIV

diagnoses based on two serial RDTs had been incorrect. Compared to other published data on RDT performance in Africa where up to 10% FP rate was reported [15–18], our FP rate appears low in this setting. This could be a signal that there is gradual improvement in the quality of HTS in the region but also represent the sampling of a relatively well-resourced public sector health system in South Africa. Of note,

although these women were enrolled in a clinical trial with rigorous quality assurance (QA) processes, their RDT-based HIV diagnoses were made in routine primary care services prior to trial screening, and thus, we do not believe the trial participation impacted on the FP estimate.

The WHO guidelines on HTS focus on the 5 C's: consent, confidentiality, counselling, correct results and connection. Within the correct result focus, appropriate use of testing algorithms and retesting before ART initiation are the two major components of ensuring accurate results. The rationale behind the sequential positive RDTs to confirm HIV diagnosis when performed correctly is that the multiplicative effect of combining highly specific RDTs should make misdiagnosis extremely rare. In the WHO laboratory evaluations, the sequential RDT approach achieved >99% positive predictive value when compared to gold standard. However, user errors such as clerical error or poor reading/interpretation can cause non-specificity across various RDTs, despite good assay performance characteristics. Recognizing this potential issue, retesting thus forms a large part of the current WHO strategy to minimize the misdiagnosis. However, many countries' HIV testing strategies still do not align with the WHO recommendations and many countries, including South Africa, do not have established retesting procedures to confirm the initial screening and confirmatory positive RDTs [10]. In our crude cost comparison, retesting using either an additional RDT or ELISA in order to mitigate FP misclassification is cost saving even if the FP rate is as low as 0.1%. In many LMIC where there are already limited resource of HIV diagnosis, limiting the ELISA confirmation to individuals with CD4 >350 can be a potential strategy which further reduce the cost by 40% while still detecting all FP RDT result. HIV programmes implementing universal ART need to identify a retesting policy that does not delay ART initiation as a matter of urgency as our data suggest that the cost associated with unnecessary lifelong ART and VL monitoring of few individuals misdiagnosed as HIV infected is substantial.

There is no doubt that improving the quality of RDTs should be a key focus of all HTS. Initiatives such as the Rapid Test Quality Improvement Initiative (RTQII) provide quality assurance support and material for proficiency testing. The footprint of these programmes span across many PEPFAR-supported countries and is a key to long-term success of HIV diagnosis in resource-limited settings. It would take a long time and much investment to roll out QA programmes to all the facilities that make use of RDTs to definitively diagnose infection. In South Africa where laboratory infrastructure is good, ELISA performed in a laboratory setting with a quality assurance programme could be invaluable. This form of retesting contributes to not only more accurate diagnoses, but can also be used as a tool to identify primary care facilities that require urgent quality improvement in their RDT programme. It is a far simpler task to roll out quality assurance among laboratories performing ELISA than to have rapid scale up of RTQII coverage of all facilities using RDT to diagnose HIV.

In 2014, the Centers for Disease Control and Prevention (CDC) changed its laboratory diagnostic algorithm to include nucleic acid amplification testing for those samples found to

be indeterminate using HIV immunoassays. Similarly, pre-ART VL testing has the potential to be used for confirmatory testing. However, our findings suggest that many women with VL <50 copies were antibody positive. There are many reasons pre-ART individuals may present as aviraemic. A detailed discussion of this phenomenon is beyond the scope of this article, but undisclosed ART use dispensed from a different facility and transient virological control are just two common causes that may confound the use of VL as a confirmatory tool [19–20]. In these aviraemic individuals, further serological testing is required, but our calculation suggests that this tandem VL-ELISA approach is 10 times more costly than simply confirming every positive RDT with ELISA alone.

There are some limitations to our study, the most important of which is the lack of detail around women's initial RDTs. Reliable documentation of whether any of the positive RDT results were "weakly reactive" could inform potential weaknesses in the current algorithm. This speaks to the fundamental issue around the general lack of formal documentation of RDT HIV diagnoses. In our laboratory confirmation testing, although Western blot was not used to confirm the cases of FP misdiagnosis, the combination of negative ELISA and nucleic acid test is highly specific. For simplicity, we assumed that all confirmation tests, including a further RDT, are 100% specific when calculating the cost of identifying a single FP. In settings where the quality assurance of RDT is poor, our approach would likely underestimate the true cost, and many misdiagnoses can go undetected. More studies with a greater health economic focus are needed to guide the retest policy of many LMIC [21]. Finally, we were not able to assess the greater societal cost of misdiagnosis or the psychosocial impact for the affected individual. Given that they are likely non-trivial, retesting and quality improvement should be a top priority in all HTS, and more resources should be dedicated to ensure that the correct results are provided in our testing facilities.

Conclusions

In summary, these results suggest that even in the setting where FP HIV RDT diagnoses are relatively uncommon, retesting with additional RDT or ELISA can be cost saving. While testing programmes based on RDT should strive for constant quality improvement, where resources permit, laboratory confirmation algorithms can be cost saving and can play an important role in strengthening the quality of HIV diagnosis in the era of universal ART.

Authors' affiliations

¹Division of Virology, Department of Pathology, University of Cape Town and National Health Laboratory Service, Cape Town, South Africa; ²ICAP, Columbia University, Mailman School of Public Health, New York, NY, USA; ³Division of Epidemiology and Biostatistics and Centre for Infectious Disease Epidemiology and Research, School of Public Health and Family Medicine, University of Cape Town, Cape Town, South Africa; ⁴College of Physicians & Surgeons, Columbia University, New York, NY, USA

Competing interests

None declared.

Authors' contributions

EJA, LM and NH conceived and designed the study and wrote the manuscript. NH, AZ and TKP analysed the results and wrote the manuscript. All authors have read and approved the final version.

Acknowledgements

We thank the MCH-ART study staff, NHLS laboratories and NHLS laboratory staff for enrolling the patients and performing the laboratory testing.

Funding

This research was supported by the President's Emergency Plan for AIDS Relief (PEPFAR) through the National Institute of Child Health and Human Development (NICHD) [Grant Number 1R01HD074558]. Additional funding comes from the Elizabeth Glaser Pediatric AIDS Foundation.

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Viewpoint

HIV misdiagnosis in paediatrics: unpacking the complexity

Emma Sacks^{1§}, Jennifer Cohn^{2,3} and Martina Penazzato⁴

[§]**Corresponding author:** Emma Sacks, Elizabeth Glaser Pediatric AIDS Foundation, 1140 Connecticut Ave NW Suite 200, Washington, DC 20036, USA. (esacks@pedaids.org)

Received 20 March 2017; Accepted 8 June 2017; Published 29 August 2017

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Introduction

Timely and accurate diagnosis of paediatric HIV infection continues to be a public health challenge. Scale up of interventions to prevent mother-to-child HIV transmission (PMTCT) has facilitated the strengthening of early infant diagnosis (EID) programmes, but more needs to be done to ensure that infants and children infected with HIV are identified in a timely manner and effectively linked to care and treatment. In particular, HIV testing of those HIV-exposed children missed by PMTCT services will require more focused attention and increasingly more innovative approaches. In addition to optimizing the appropriate use of HIV testing along the paediatric diagnostic cascade, it is critical to minimize misdiagnosis to prevent mortality resulting from false negative testing, unnecessary lifelong treatment in HIV-uninfected children who receive a false positive diagnosis and potential psychosocial sequelae resulting from misdiagnosis.

While it is true that children face many of the same issues with misdiagnosis as adults, including the accuracy of currently available technologies, cross-contamination or mislabelling of specimens, inadequate adherence to testing guidelines and algorithms, and issues with interpretation of test results, a number of issues specific to paediatric testing need to be considered. Transmission dynamics, natural history and decay of maternal antibodies result in additional complexities that may affect interpretation and management of test results. Both virological and serological test performance may also be affected by the timing of testing and exposure to antiretroviral (ARV) drugs taken by the mother and/or the infant. Overall, the rapid progression of disease among untreated, infected children further highlights the importance of minimizing misdiagnosis and ensuring prompt identification of HIV-infected infants and children.

Unique challenges of HIV testing in infants and children

There are a number of challenges across the paediatric HIV diagnostic cascade for children which relate to the use of virological or antibody testing for the purpose of assessing

HIV exposure and/or identifying HIV infection (Table 1). Virological testing is required to ascertain HIV status in infants and children below 18 months. A number of molecular testing platforms are currently available for EID, including conventional testing and the newer point-of-care (POC) platforms. Misdiagnosis, with false negatives, false positives and indeterminate results, has been reported in a number of settings [1–3]. False negative results are of greatest concern in terms of risk to the infant but little is known about the implications of indeterminate results. The performance of these nucleic acid tests (NATs) varies and, especially in infants, could depend on a number of factors. Transmission and viral dynamics are the most important, particularly as more effective PMTCT interventions are being scaled up. Timing of testing plays an obvious but important role: intrauterine transmissions can be identified with virological testing as early as birth, while intrapartum and early breastfeeding transmissions require NAT at a later point, usually at age 4–6 weeks.

Exposure to maternal ARV or postnatal prophylaxis (particularly when multi-drug or prolonged) is another potential factor that may reduce the viral load in an infected infant and lower the sensitivity of the test. However, to date, evidence is sparse and conflicting, with some studies documenting a delay in detectability [1,4] and others confirming identification of virus load as low as 40 copies/ml [5], even when POC assays are used. Evidence generated after the WHO guidelines were issued demonstrate good performance at birth, as well as 6 weeks, with sensitivity and specificity ranging from 93.3–100% and 99–100%, respectively [5–10]. As suggested by Technau et al. in this supplement, exposure to ARVs may also increase the number of indeterminate results (defined as a detected target with a cycle threshold (Ct) greater than 33 on a quantitative PCR) and these infants are often diagnosed as HIV-infected at later testing [11]. These factors make it critical that infants with negative NAT are retained in the postnatal period and offered later testing to fully rule out infection as well as to offer continued support in preventing transmission through breast milk.

Use of rapid antibody diagnostic tests (RDTs) presents a separate set of challenges regarding the potential for

Table 1. Potential issues and mitigating actions for misdiagnosis in paediatric HIV testing

| Potential issues | | Mitigating actions |
|------------------------|---|---|
| <i>False positives</i> | | |
| NAT | Reduction in positive predictive value with decreasing transmission rates | Confirmatory testing upon initial positive NAT (with conventional or POC NAT) |
| Antibodies | Patterns of maternal antibody decay and infant antibodies production | Ensuring appropriate timing and interpretation of RDT results by health care workers |
| <i>False negatives</i> | | |
| NAT | Transmission dynamic and timing of infection Exposure to ARV (particularly enhanced prophylaxis) | Ensure implementation of WHO testing algorithm Ensuring ascertainment of final diagnosis at the end of breastfeeding Gather additional data on impact of ARV exposure on NAT results |
| Antibodies | Patterns of maternal ab decay and infant antibodies production Immunological suppression resulting in lack of antibody response Seroreversion as a result of early ART initiation | Ensure implementation of WHO testing algorithm Ensuring ascertainment of final diagnosis at the end of breastfeeding No repeat testing once diagnosis is confirmed and ART is started Gather evidence on false negative RDT for asymptomatic infants with severe immunosuppression |

paediatric misdiagnosis. RDTs are used both to document HIV exposure in infants as well as to diagnose HIV after 18 months of age. A recent review that informed WHO guidelines highlights the potential for false negative results when infants are tested for exposure after 4 months, with sensitivity as low as 60% [12]. This phenomenon results from the decay of maternal antibodies below the threshold of detectability, highlighting the importance of testing the mother whenever possible or using cautious interpretation of any negative results in infants tested after 4 months whose mother's status is unknown. Use of RDT for diagnosis requires infant production of antibodies, which relates to time of transmission and may be attenuated in infants that are already significantly immunosuppressed as a result of HIV infection. While a negative RDT after 9 months is considered reliable to exclude current HIV infection, it does not provide final diagnosis. This is particularly true for infants who are ill and who may have a false negative RDT, and for those still at risk due to breastfeeding [13]. Similar to NAT, repeat longitudinal testing is critical to ensure that risk of misdiagnosis is minimized.

Evolving scenarios and new issues

Scale up of PMTCT and early ART for infants and children will likely add additional layers of complexity. First, as PMTCT decreases perinatal transmission, the positive predictive value of a single test will continue to decrease, resulting in potentially more false positive results, highlighting the importance of ensuring confirmatory testing is provided. Recent cost-effective analysis indicates that confirmatory testing is cost-effective and its value increases as transmission rates decrease [14]. However, repeat testing should not delay ART initiation and treatment should be started upon the first positive result. POC may be useful in providing a more rapid confirmation of a positive initial test.

POC NAT is a new technology and its benefits over conventional testing, including more rapid turnaround time and increased percentage of HIV-infected children initiated on ART, suggest that, at current prices, it may be cost-effective as compared to conventional NAT [15]. Studies have begun to formally assess the cost-effectiveness of POC versus conventional NAT for EID. Additionally, the possibility of cost containment with bulk purchasing of equipment and materials, multiplexing (for EID, viral load and tuberculosis), and the creation of testing and transport networks to share machines, these platforms may be shown to be a worthwhile investment.

Second, children that are started on treatment early in life may never develop HIV antibodies and have false negative results on RDT as shown in Ferrand in this supplement [16]. A number of studies have documented that this phenomenon is more likely when infants are started on ART in the first months of life [17,18], but false negatives may also occur when ART is started later, particularly when oral RDTs are used. This has the potential to generate confusion among providers and the family and may present the dilemma of interrupting ART to see if the child is truly infected. While NAT may help to resolve some of these cases, false negative results may also occur with NAT especially if the child is fully suppressed on ART [19]. Therefore, ensuring that an accurate diagnosis is conducted with specimens collected before ART is started will be critical. This will also require appropriate messaging to caregivers regarding the potential for false negatives in children treated with early ART.

Overall, there is almost no qualitative research on issues regarding misdiagnosis of HIV testing in children. A paucity of information exists about caregivers' understanding of discordant results, barriers to or information needed for informed decision-making about potential lifetime treatment initiation for infants, and the social consequences, including issues around disclosure to

family and friends, of indeterminate and delayed results. Little is understood about practical or behavioural implications of changing diagnoses for families who are affected, but even with limited evidence, given historical mistrust around HIV, programmes should be especially sensitive to the potential for misdiagnosis to erode trust between families and the health system and should proactively express the possibilities and mitigation strategies for misdiagnosis to all involved.

Conclusions

In the current HIV response, the primary issue in paediatric HIV diagnosis is ensuring the scale up of timely HIV testing in infants and children, but misdiagnosis should not be forgotten. The causes of misdiagnosis in children are complex, yet there is reason for optimism. Complex viral dynamics, coupled with the high mortality for untreated, HIV-infected infants, make it even more critical to ensure children complete the entire diagnostic cascade, providing multiple opportunities to diagnosis HIV-infected children. New developments in diagnostic technologies, such as POC NAT, are changing the landscape and improving timely patient access to appropriate diagnostic modalities. Strategies to improve accuracy of diagnosis, as well as timely receipt of results and follow-up, are paramount to improving care and reducing HIV-related mortality that disproportionately affects HIV-exposed infants.

In summary, key actions to minimize misdiagnosis include (1) ensuring that all HIV-exposed infants and children are retained and complete the WHO-recommended testing cascade until final diagnosis is ascertained after completion of breastfeeding; (2) confirmatory testing is provided to any child who has a positive initial NAT; (3) once diagnosis is confirmed and ART is started, further testing is not recommended and, if conducted, negative results should be interpreted with extreme caution; and (4) clear messaging and community awareness about the importance of the entire EID cascade is critical. These actions need to be considered in the context of a more strategic integration of paediatric HIV testing into the wider child survival platform to mainstream and expand access to quality and timely HIV testing.

Authors' affiliations

¹Department of Research, Elizabeth Glaser Pediatric AIDS Foundation, Washington, DC, USA; ²Department of Innovation and New Technology, Elizabeth Glaser Pediatric AIDS Foundation, Geneva, Switzerland; ³Division of Infectious Diseases, University of Pennsylvania School of Medicine, Philadelphia, PA, USA; ⁴HIV Department, World Health Organization, Geneva, Switzerland

Competing interests

The authors have no conflicts of interest to declare.

Disclaimer: The conclusions and opinions expressed in this article are those of the authors and do not necessarily reflect those of the World Health Organization.

Funding

There is no funding source.

Authors' contributions

ES, JC and MP conceived of the idea, researched, wrote and edited the manuscript. All authors have read and approved the final version.

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Research article

HIV point of care diagnosis: preventing misdiagnosis experience from a pilot of rapid test algorithm implementation in selected communes in Vietnam

Van Thi Thuy Nguyen^{1§}, Susan Best², Hong Thang Pham³, Thi Xuan Lien Troung⁴, Thi Thanh Ha Hoang³, Kim Wilson², Thi Hong Hanh Ngo³, Xuan Chien⁵, Kim Anh Lai⁶, Duc Duong Bui⁷ and Masaya Kato¹

[§]Corresponding author: Van Thi Thuy Nguyen, World Health Organization, Vietnam Country Office, Hanoi, Vietnam. Tel: +844 3850 0314. (nguyenva@who.int)

Abstract

Introduction: In Vietnam, HIV testing services had been available only at provincial and district health facilities, but not at the primary health facilities. Consequently, access to HIV testing services had been limited especially in rural areas. In 2012, Vietnam piloted decentralization and integration of HIV services at commune health stations (CHSs). As a part of this pilot, a three-rapid test algorithm was introduced at CHSs. The objective of this study was to assess the performance of a three-rapid test algorithm and the implementation of quality assurance measures to prevent misdiagnosis, at primary health facilities.

Methods: The three-rapid test algorithm (Determine HIV-1/2, followed by ACON HIV 1/2 and DoubleCheckGold HIV 1&2 in parallel) was piloted at CHSs from August 2012 to December 2013. Commune health staff were trained to perform HIV testing. Specimens from CHSs were sent to the provincial confirmatory laboratory (PCL) for confirmatory and validation testing. Quality assurance measures were undertaken including training, competency assessment, field technical assistance, supervision and monitoring and external quality assessment (EQA). Data on HIV testing were collected from the testing logbooks at commune and provincial facilities. Descriptive analysis was conducted. Sensitivity and specificity of the rapid testing algorithm were calculated.

Results: A total of 1,373 people received HIV testing and counselling (HTC) at CHSs. Eighty people were diagnosed with HIV infection (5.8%). The 755/1244 specimens reported as HIV negative at the CHS were sent to PCL and confirmed as negative, and all 80 specimens reported as HIV positive at CHS were confirmed as positive at the PCL. Forty-nine specimens that were reactive with Determine but negative with ACON and DoubleCheckGold at the CHSs were confirmed negative at the PCL. The results show this rapid test algorithm to be 100% sensitive and 100% specific. Of 21 CHSs that received two rounds of EQA panels, 20 CHSs submitted accurate results.

Conclusions: Decentralization of HIV confirmatory testing to CHS is feasible in Vietnam. The results obtained from this pilot provided strong evidence of the feasibility of HIV testing at primary health facilities. Quality assurance measures including training, competency assessment, regular monitoring and supervision and an EQA scheme are essential for prevention of misdiagnosis.

Keywords: HIV testing; rapid test; algorithm; decentralization; misdiagnosis; quality assurance; Vietnam

Received 16 December 2016; Accepted 19 June 2017; Published 29 August 2017

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Introduction

There were an estimated 260,000 people living with HIV (PLHIV) in Vietnam at the end of 2015, and the HIV epidemic is concentrated in key populations – people who inject drugs (PWID), female sex workers (FSW) and men who have sex with men (MSM) [1]. Among them, 106,373 received anti-retroviral therapy (ART) accounting for 42% of PLHIV [1]. More than 50% of PLHIV have not accessed ART or have not been diagnosed. Although, HIV testing and counselling (HTC) has been expanded, especially in high and medium burden provinces, the access by key populations to this service is still limited. In 2015, the percentage of PWID,

MSM and FSW who received an HIV test in the preceding 12 months was 30%, 32% and 41%, respectively [1].

Several reasons may contribute to low uptake of HTC among key populations and pregnant women including stigma, discrimination, and inconvenience or difficulties in reaching HTC facilities. In Vietnam, HTC had been mainly available at the district level facilities via client-initiated (e.g. voluntary HTC services) and provider-initiated approaches (e.g. antennal care services). To confirm a blood specimen as HIV positive, one showing reactivity in the screening test needs to be sent to a provincial-level laboratory that is authorized by the Ministry of Health

(MoH) as a confirmatory testing laboratory. As a consequence, turnaround time for the results may take from one week to four weeks especially in remote and mountainous provinces. Due to limited services and long turnaround time for results and other reasons such as poor linkages between HTC and ART services sites and lack of effective monitoring of referral services, loss to follow-up between diagnosis and enrolment in care has been a significant programmatic issue in HIV cascades in Vietnam.

In the past years, HIV diagnostic rapid tests have become widely available and have good performance compared with EIA [2,3]. This allows decentralization of HIV testing from provincial and district laboratories to primary health-care facilities. Decentralization and integration of HTC to lower-level healthcare facilities could facilitate access especially for key populations in remote and mountainous provinces. To improve access to HTC, HIV services, including same-day test results, need to be more accessible to key populations.

Vietnam is one of the few countries in the world piloting the Treatment 2.0 initiative launched by WHO and UNAIDS in 2011 [4]. One of the five pillars of this initiative is using point of care (POC) diagnosis. The pilot study was implemented in two provinces, one in the urban area of the south (Can Tho city) and one in the mountainous area of the north (Dien Bien province). Within the scope of the pilot, POC HIV testing was introduced and promoted through decentralizing and integrating HIV testing into commune health stations (CHSs). This innovative model is expected to facilitate early diagnosis and early access to ART for key populations. This pilot was designed to demonstrate and assess the performance of decentralized HIV screening and confirmatory testing, and validation in the field, of a rapid testing algorithm. This paper is based on the data reported during the Treatment 2.0 pilot.

Methods

Description of the pilot

Pilot sites

The pilot was carried out in 21 communes in seven districts: four districts in Dien Bien province (Dien Bien city, Dien Bien district, Muong Ang and Tuan Giao) and three districts in Can Tho city (Ninh Kieu, O Mon and Vinh Thanh) in August 2012. This study was part of the pilot implementation which was evaluated from August 2012 to December 2013.

Selection of rapid test algorithm

Based on the results of the evaluation of HIV test kits conducted by the National Institute of Hygiene and Epidemiology (NIHE) in 2011 [5], with technical assistance from the National Serology Reference Laboratory, Australia, the national technical working group selected three rapid tests to combine into an algorithm. The rapid tests included Determine HIV-1/2 (Alere, Japan) as the screening test and the ACON HIV 1/2 (ACON Laboratories, Sadiago, United States) and DoubleCheckGold HIV 1&2 (Orgenics Ltd.,Yavne, Israel) as the second and the third assays (sensitivity and specificity of these three rapid tests are presented in Table 1). The

Table 1. Sensitivity and specificity of the three rapid tests based on results of the national evaluation of HIV test kits

| Test kits | Sensitivity (95% CI) | Specificity (95% CI) |
|-------------------------|----------------------|----------------------|
| Determine HIV-1/2 | 99.50 (98.94–100.0) | 95.74 (94.12–97.36) |
| ACON HIV 1/2 | 99.50 (98.94–100.0) | 100.0 (100.0–100.0) |
| DoubleCheckGold HIV 1&2 | 99.00 (98.20–99.80) | 99.75 (99.35–100.0) |

algorithm was chosen according to WHO recommendations for developing an HIV testing algorithm for diagnosis and in line with recommended sensitivities and specificities for screening and confirmatory tests [6,7].

Determination of serostatus at commune health station

At commune health stations, trained staff provided pre-test counselling to clients and verbal consent was obtained. Between August 2012 and July 2013, venous blood was taken and plasma was collected for HIV testing. However, from August 2013 commune health staff were trained on performing finger prick blood collection and henceforth, capillary whole blood was used for the screening test. Clients whose specimens were reactive on the Determine had venous blood collected from which the plasma was extracted and sent to the Provincial Confirmatory Laboratory (PCL) for confirmation of the HIV status of such specimens, in line with the Vietnam MoH regulation on HIV testing.

For the purpose of validating the algorithm, plasma was also used for further testing with the ACON and DoubleCheckGold in parallel at the CHSs. Results of the tests were recorded in a logbook along with an overall interpretation based on the results of all tests performed. Specimens that gave non-reactive results on Determine HIV1/2 were recorded as negative; specimens that were reactive with all three tests were recorded as HIV positive; specimens that were reactive with one or two tests were recorded as indeterminate at the CHSs (Figure 1). Results for specimens showing reactivity on Determine HIV1/2 were not returned to the clients until the confirmatory test result was confirmed by the PCL. Worksheets were used to record results when performing the tests and the results of tests were then recorded in a logbook and the worksheets were sent to the reference laboratories along with the sample.

Validation and confirmation of HIV testing at provincial confirmatory laboratories

All specimens that were positive or indeterminate were sent to the supervising PCL for confirmation. In addition, specimens that were negative in the first three months of the pilot implementation were sent to the supervising PCL for validation. Algorithms used at both PCLs included 4th

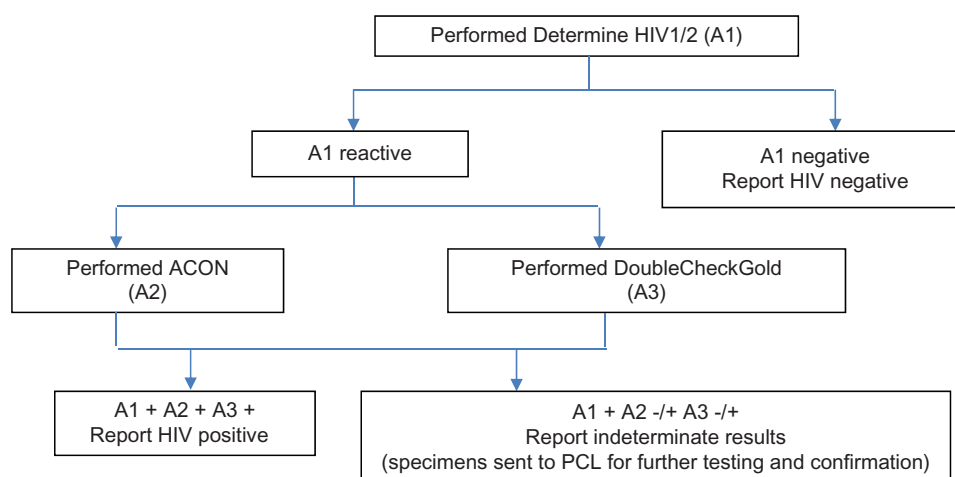


Figure 1. HIV testing algorithm performed at commune health stations.

generation enzyme immunoassays (EIA), either Murex HIV Ag/Ab Combination (DiaSorin, Italy) [Murex] or Genscreen Ultra HIV Ag/Ab EIA (Bio-Rad, France) [Genscreen] as assay one (A1), a particle agglutination assay Serodia HIV1/2 (Fujirebio, Japan) as assay two (A2), and a rapid test either SD Bioline HIV-1/2 3.0 (Alere, Korea) or Determine HIV-1/2 as assays three (A3). For the purposes of the validation, specimens negative on Determine HIV1/2 at the CHSs were retested by Murex or Genscreen EIA. Specimens that gave negative results on EIA were confirmed as negative. Specimens that were reactive in all three tests of the respective PCL algorithm were confirmed as positive. If

specimens reactive in one or two tests of the respective PCL algorithm they were given a status of “indeterminate” and the clients were requested to return into two weeks to retest (Figure 2).

Sensitivity and specificity of three rapid test algorithm was calculated based on the following formula:

$$\text{Sensitivity} = \frac{\text{True positivity}}{\text{True positivity} + \text{false positivity}} \times 100$$

$$\text{Specificity} = \frac{\text{True negativity}}{\text{True negativity} + \text{false negativity}} \times 100$$

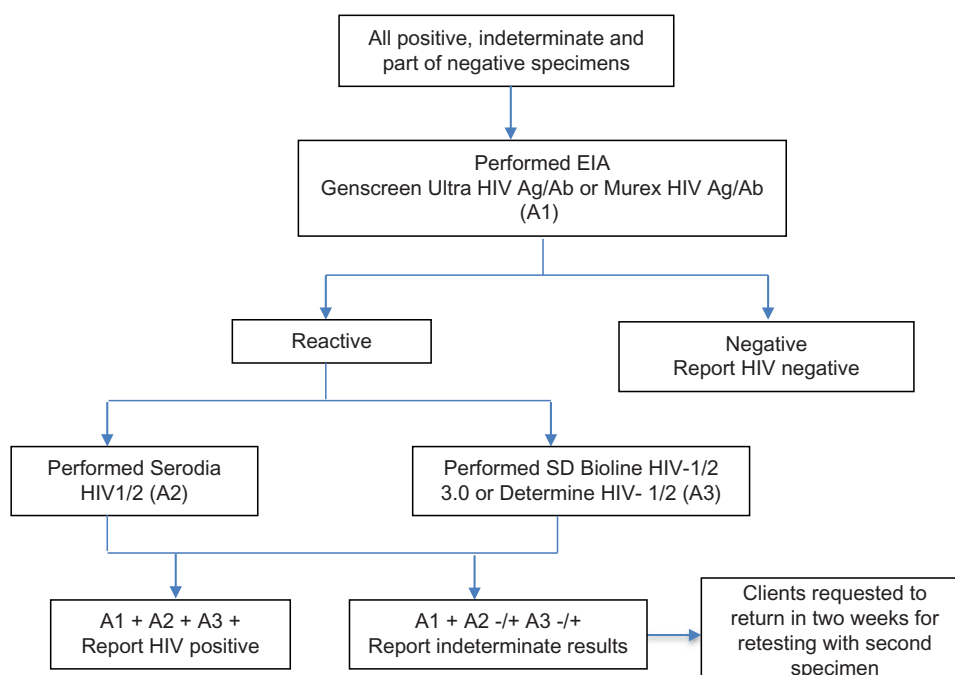


Figure 2. HIV testing algorithm performed at provincial confirmatory laboratory.

Result communication

Clients were asked to wait for 30 min to receive results or return any time within that day according to the client's preference. If the screening test was negative, commune health staff provided the test result and post-test counselling. In the case of reactive results, clients were counselled on the need for confirmatory testing. An appointment was made to come back to receive the confirmatory test result and referral to care if necessary. During the validation process, effort was made to expedite the confirmatory testing to ensure the clients receive the confirmatory results within 3–5 days. Prevention interventions were also encouraged during pre- and post-test counselling of the clients.

Quality assurance

To ensure the quality of the HTC, a range of activities were undertaken including training, competency assessment, field technical assistance, supervision and monitoring and external quality assessment (EQA). Prior to the implementation of the pilot study, staff from the 21 CHS undertook a 5-day training course on HTC, delivered by trainers from NIHE and the Pasteur Institute (PI) in Ho Chi Minh City and provincial AIDS centres (PACs). In addition, a coded set of specimens was sent to each CHS for final assessment of staff competence. Only commune health staff who received certificates from training and were assessed as competent were assigned to perform HIV testing. Regular supervision and monitoring at CHS were conducted by staff from PACs and district health centres with technical assistance from NIHE, PI Ho Chi Minh City and WHO. In addition, these communes also participated in an EQA scheme. EQA panels which included 10 coded specimens were sent to these communes by NIHE twice a year to monitor the quality of HIV testing.

Data collection and data analysis

Data on HIV testing were collected using a customized form including patient code, address, sex, year of birth and results of HIV testing at the commune and provincial levels. In addition, information on a client's self-reported HIV risk was recorded, as PWID, MSM, sex workers, sexual partners of PWID or PLHIV and pregnant women in HTC logbooks at the CHSs and confirmatory laboratories. Descriptive analysis was conducted. Sensitivity and specificity of the rapid testing algorithm were also calculated.

Ethical approval

The pilot was implemented following the Decision of Viet Nam Ministry of Health (Decision 1039, dated April 3 2012).

Results

Characteristics of clients who received HTC at CHS

Between August 2012 and December 2013, a total of 1,373 people received HTC at CHS including 938 pregnant women (68.3%), 137 PWID (10.0%), 12 FSW (0.9%), 170 partners of PWID or partners of PLHIV (12.4%) and 116 others (8.4%). Female clients accounted for 84% and most of them were

Table 2. Characteristics of clients who received HTC at 7 districts between August 2012 and December 2013

| Characteristics | Frequency (N = 1373) | Percentage |
|---|-------------------------|------------|
| Age (mean±SD 28.6 ± 7.7; range 5 – 66) | | |
| <15 | 5 | 0.36 |
| 15–49 | 1336 | 97.3 |
| >49 | 32 | 2.3 |
| Sex^a | | |
| Female | 1149 | 83.7 |
| Male | 223 | 16.3 |
| Population groups | | |
| Pregnant women | 938 | 68.3 |
| People who inject drugs (PWID) | 137 | 10.0 |
| Female sex workers | 12 | 0.9 |
| Partners of PLHIV or PWID | 170 | 12.4 |
| Other | 116 | 8.4 |
| Residency | | |
| <i>Dien Bien province</i> | | |
| Dien Bien district | 386 | 28.1 |
| Dien Bien city | 162 | 11.8 |
| Muong Ang district | 214 | 15.6 |
| Tuan giao district | 108 | 7.9 |
| <i>Can Tho province</i> | | |
| Ninh Kieu district | 230 | 16.8 |
| O mon | 168 | 12.2 |
| Vinh Thanh district | 105 | 7.6 |

^aOne missing value

pregnant women (82%) (Table 2). Eighty people were diagnosed with HIV infection (5.8%) including 6 pregnant women. All of these clients were followed and 65 were enrolled in care (81.2%), 5 died, 3 did not comeback for results, 4 moved out of province for work, 1 was sent to prison and 2 were lost-to-follow-up.

Testing algorithm validation

Of the 1,244 specimens that gave a negative screening test result at the CHSs, 755 were sent to the PCL for validation testing along with all 129 specimens that were reactive with the screening test using Determine (Figure 3). At the PCLs, all negative specimens sent from CHS were confirmed as negative by Genscreen or Murex EIA. All 80 specimens recorded as positive at CHS using the three rapid test algorithm were confirmed as positive by the algorithm in use at the supervising PCL (Table 3). Forty-nine specimens that were reactive with Determine but negative with ACON and DoubleCheckGold at the CHSs were confirmed negative by the PCL (Table 4). Based on the results in Table 3, sensitivity and specificity of this rapid test algorithm was calculated as below:

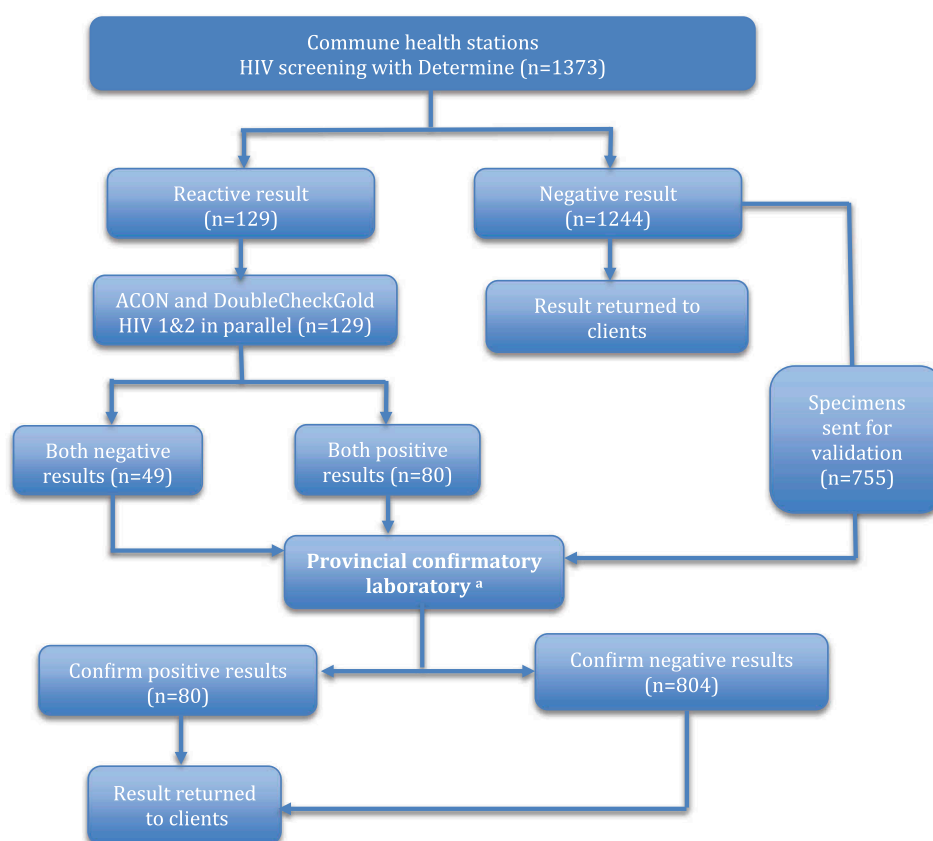


Figure 3. HIV testing validation procedure.

^a algorithm used at confirmatory testing: Murex HIV Ab/Ag Combination or Genscreen Ultra HIV Ag-Ab, Serodia HIV1/2 and SD Bioline HIV-1/2 3.0 or Determine HIV-1/2

Table 3. Comparison of HIV test results using the rapid HIV testing algorithm at CHSs with test results using an ELISA-based testing algorithm at the provincial confirmatory laboratories^a

| Testing results at CHSs using three rapid test algorithm | Results at provincial confirmatory laboratory (considered to be the gold standard) | | |
|--|---|------------|------------|
| | Positive | Negative | Total |
| HIV positive (reactive with all three rapid tests) | 80 | 0 | 80 |
| HIV negative (negative with Determine) | 0 | 755 | 755 |
| Total | 80 | 804 | 884 |

^a This table excluded 49 specimens which had indeterminate results (reactive with Determine but negative with ACON and DoubleCheckGold)

Sensitivity = $80 / (80 + 0) * 100 = 100\%$;

Specificity = $755 / (0 + 755) * 100 = 100\%$

the second round 20/20 provided accurate results. (One CHS did not submit their results to NIHE for round 2).

Performance of pilot sites in external quality assessment schemes

All 21 CHSs participated in EQA provided by NIHE. In 2013, 21 CHSs received two rounds of EQA panels. In the first round, 20/21 CHS submitted accurate results whereas in

Discussion

At the time of this pilot study, HIV confirmatory testing in Vietnam was still centralized at provincial health facilities and HIV screening was only available at the district

Table 4. Rate of falsely reactive Determine test results by risk group

| Testing populations | False reactive | % |
|---------------------------|----------------|-------|
| Pregnant women | 31 | 63.3 |
| PWID | 4 | 8.2 |
| Partners of PLHIV or PWID | 10 | 20.4 |
| Other | 4 | 8.2 |
| Total | 49 | 100.0 |

facilities. The national HIV testing policy at that time required that the testing algorithm to confirm a specimen as HIV positive included an EIA. As a result, only provincial laboratories, where the equipment and expertise to perform an EIA were available, provided confirmatory HIV testing services. Limited availability of screening and confirmatory HIV testing services and the need for an EIA for confirmatory testing contributed to low HTC uptake among key populations, long turnaround time for test results and loss of clients to follow-up after the HIV testing. For instance, in mountainous remote settings, less frequent specimen transportation from the district to the PCL contributed to increased turnaround times. A study in one mountainous province found that it took 37 days on average (ranged from 6–131 days) from the time HIV was screened at HIV screening sites until the sites received confirmatory results from PCL [8]. Similarly, in PCLs with low throughput, a requirement to test the specimens in batches using EIAs to maximize efficiency caused unacceptable delays in turnaround time.

This pilot study demonstrated a feasible and sustainable model for decentralization of HIV confirmatory testing from the provincial level down to the commune level. In the current health system in Vietnam, the CHS is the grass root level of healthcare. Prior to the pilot study, commune health staff provided primary healthcare for people living in their commune. However, HIV services were not provided at the commune level. Thus, in geographically difficult settings such as Dien Bien province, access to HIV services including HTC was very challenging for many people. In this pilot study, integration of HIV testing into the primary healthcare system brought HTC services close to peoples' homes, which facilitated better access to HTC. The Treatment 2.0 evaluation report strongly suggested high acceptability and appreciation of PLHIV for being able to access HTC service at CHS due to its convenience and time-saving (unpublished data). In addition, using the existing healthcare system to provide HIV testing services is critical for Vietnam to sustain its HIV programme in context with the decline in funding from external sources. Following this pilot, Vietnam has been piloting "test for triage" recommended by WHO 2015 consolidated guidelines on HIV testing services [9] to further decentralize HTC services at the community level. In this pilot, village health workers and peer educators in selected communes were trained to provide HIV screening tests and linkage to care.

The results will be published in a separate paper. In addition to improving access to HIV testing for key populations, access to HIV testing among pregnant women was also increased since both HTC and ANC services were offered at CHS. Vietnam is aiming to eliminate mother to child transmission of HIV by 2020 [10] and this target can only be achieved if at least 90% of pregnant women know their HIV status. Although it will need investment from the government, it has been shown to be cost-effective even in low prevalence setting [11].

The results from this pilot study also suggested that an appropriately validated rapid testing algorithm, the provision of training and the access to quality assurance programmes can be used at primary healthcare settings allowing confirmation of HIV positive results without compromising the accuracy of HIV testing. The data from this pilot study has shown consistent results between the rapid test algorithm performed by commune health staff and the more sophisticated algorithms used in provincial laboratories. This suggests that it is possible to decentralize HIV confirmatory testing to commune health facilities in Vietnam to facilitate early access to diagnosis and treatment for PLHIV, their partners, key populations and pregnant women. The evaluation of the Treatment 2.0 pilot data, revealed that people diagnosed with HIV at communes in Dien Bien started ART at a higher CD4 count (median 294 cells/mm³) than those who were diagnosed at district facilities (median 88 cells/mm³) [12]. Experiences in other countries also indicate that using a rapid testing algorithm could optimize HTC service delivery and improve linkage to care [13,14].

This pilot also mobilized and trained village health workers and peer educators to reach out to key populations and facilitate linkage between diagnosis, care and treatment. Although more than 80% of people diagnosed with HIV were successful linked to OPC for care and treatment, five people died during this pilot suggesting late diagnosis and treatment need to be addressed by making testing more accessible and strengthening linkage between diagnosis and treatment and care to ensure people diagnosed with HIV received ART in timely manner. Additionally, a mechanism for tracking clients' needs to be developed to follow-up with clients who do not return for their results.

The study findings suggested the rapid HIV testing algorithm could be applicable to larger programmes with consideration to establishing a functioning system to ensure a high quality of testing and minimize misdiagnosis. The fact that no discordant results were identified in the pilot study is most likely the result of well trained staff, availability of regular technical support and supervision of testers, participation in an EQA scheme and use of a well validated testing algorithm. It was also noted that one of 21 piloting CHS reported one or more aberrant results in one EQA round and another CHS did not report results for one EQA round. This suggested incorrect result could happen at commune facilities and thus regular technical assistance and monitoring is required to ensure quality assurance measures are effective. In this pilot, with strong supervision systems in place, the CHS that reported an aberrant result in EQA was provided with

assistance to rectify the problem. In addition, verification of HIV status before ART initiation should be considered by policy makers especially in the context of expansion of decentralization of HIV confirmatory testing to primary healthcare facilities to prevent misdiagnosis and mistreatment as recommended by WHO 2016 [15]. The testing procedure implemented in this pilot was slightly different from the WHO recommended strategy for HIV diagnosis. In this pilot, assay 2 and 3 were performed in parallel if assay 1 was reactive. The reason for this is that this is the first time ever that HIV testing had been introduced at the commune health stations, who had no previous testing experience. Thus, we try to simplify the testing procedure by performing the second (A2) and third (A3) assays in parallel. Commune health staff were trained to record a positive result only if all three assays were reactive. Any discordant results between the three assays were recorded as indeterminate and required confirmation by the PCL.

In a larger Nigerian study, where a two rapid test algorithm was used, 6% of HIV test results were reported falsely positive. The authors suggested potential risks for errors including lack of a quality management system in these laboratories, inconsistent or incorrect use of rapid test algorithms; incorrect interpretation of the weakly-positive test lines and non-usage of a third test [16]. At a WHO meeting held in Geneva in March 2016 discussing the social, public health, human rights, ethical and legal implications of misdiagnosis of HIV status, WHO and the US Centers for Disease Control reported a range of 0.7–10.5% misdiagnosis of HIV-positive status from programme settings and external quality assessment schemes [17]. Common issues accounting for misdiagnosis raised in this meeting included difficulty in reading weakly reactive lines, not using a validated national testing algorithm or a WHO recommended testing strategy, poor training, support and supervision of testers, specimen mix-up and not following standard operating procedures [18–20]. Therefore, to expand community-based HIV testing in Vietnam using a rapid testing algorithm, a quality management system needs to be well established within the programme including a standardized training curriculum, standard operating procedures, technical assistance and supervision and an EQA scheme. Furthermore, the national testing algorithm needs to be implemented at all testing sites.

This pilot study has several limitations. First, this pilot was designed as a demonstration pilot to assess feasibility including quality of HIV POC diagnosis at commune health facility and not designed for validation of the testing algorithm, thus sample size may not be large enough to conclude sensitivity and specificity of the testing algorithm. The pilot was implemented in only two provinces, one mountainous province in the North West and one city in Mekong River Delta. Thus, in other provinces with relatively different culture, geographical characteristics and level of stigma and discrimination, willingness to access HTC at commune health stations among key populations may not be the same and interventions need to be tailored to meet the needs of these

key populations to enhance the efficiency of HIV POC diagnosis.

Conclusions

Decentralization of HIV confirmatory testing to CHS is feasible in Vietnam. The results obtained using the rapid testing algorithm provided strong evidence on the feasibility of HIV testing at primary healthcare settings. Quality assurance measures including training, competency assessment, regular monitoring and supervision and an EQA scheme are essential to ensure accurate test results. This pilot made an important contribution by providing data that convinced the Ministry of Health to amend its policy to decentralize HIV testing services and to ensure high-quality HIV testing is available at primary healthcare facilities.

Authors' affiliations

¹World Health Organization, Vietnam Country Office, Hanoi, Vietnam; ²Australian National Serology Reference Laboratory, Melbourne, Australia; ³HIV Department, National Institute for Hygiene and Epidemiology, Hanoi, Vietnam; ⁴Pasteur Institute, Ho Chi Minh City, Vietnam; ⁵HIV Laboratory, Dien Bien Provincial AIDS Centre, Vietnam; ⁶Can Tho Preventive Medicine Centre, Can Tho City, Vietnam; ⁷Viet Nam Authority for HIV/AIDS Control, Ministry of Health, Hanoi, Vietnam

Competing interests

The authors have no competing interests to declare.

Authors' contributions

All authors have read and approved the final manuscript. VTTN: designed the demonstration of decentralized HIV testing services, promoted access to hard-to-reach areas, while ensuring the quality of HIV testing. Leading the process of data collection, data analysis, drafted the manuscript and co-ordinated the review of the manuscript with other co-authors
SB: provided technical assistance in development of testing algorithm; provided significant technical inputs and reviewed the manuscript
PHT: provided technical assistance in development of testing algorithm, training and monitoring the implementation, and reviewed the manuscript
HTH: provided technical assistance in development of testing algorithm, training and monitoring the quality of testing, and reviewed the manuscript
TXL: provided technical assistance in development of testing algorithm, training and monitoring the implementation, and reviewed the manuscript
NTHH: provided technical assistance in training and monitoring the implementation, and quality assurance
KW: provided technical assistance in development of testing algorithm, training materials and reviewed the manuscript
HXC: assisted in implementation and data collection
LKA: assisted in implementation and data collection
BDD: provided coordination support in implementation of Treatment 2.0 including point-of-care diagnosis component
MK: provided TA in designing the demonstration of decentralized HIV testing services, promoting access to hard-to-reach areas, while ensuring the quality of HIV testing. Provided technical advices in data analysis and took part in the critical review of manuscript

Acknowledgements

The authors would like to thank laboratory staff and health staff at Can Tho and Dien Bien Provincial AIDS Centre, district and commune health staff, village health workers and peer educators in Dien Bien city, Dien Bien, Tuan Giao, and Muong Ang districts (Dien Bien province) and Ninh Kieu, O Mon and Vinh Thanh (Can Tho city) who participated in this pilot study. The authors also thanks to MAC AIDS Foundation for funding this pilot.

Funding

No funding was required for development of the manuscript.

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Email : editorial@ijasociety.org

Website: <http://www.ijasociety.org>

eISSN: 1758-2652

Publisher

International AIDS Society

Avenue de France 23

1202 Geneva, Switzerland

Tel: +41 (0) 22 710 0800

Email: info@iasociety.org

Website: <http://www.iasociety.org>

Indexing/abstracting

The *Journal of the International AIDS Society* is indexed in a variety of databases including PubMed, PubMed Central, MEDLINE, Science Citation Index Expanded and Google Scholar. The journal's impact factor is 6.296 (2016 Journal Citation Reports® Science Edition - a Clarivate Analytics product).

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