Open Forum Infectious Diseases

BRIEF REPORT

Reflex Human Immunodeficiency Virus (HIV) Type 1 RNA Testing Enables Timely Differentiation of False-Positive Results From Acute HIV Infection

Christopher Kaperak,[1] Dylan Eller,[2,3] Samantha A. Devlin,[2,3] André Hall,[2,3] Jessica Schmitt,[2,3] Eleanor E. Friedman,[2,3] Kathleen G. Beavis,[4] Kimberly A. Stanford,[5] David Pitrak,[2,3] and Moira C. McNulty[2,3]

[1]Department of Medicine, University of Chicago Medicine, Chicago, Illinois, USA, [2]Section of Infectious Diseases and Global Health, University of Chicago, Chicago, Illinois, USA, [3]Chicago Center for HIV Elimination, University of Chicago, Chicago, Illinois, USA, [4]Department of Pathology, University of Chicago, Chicago, Illinois, USA, and [5]Section of Emergency Medicine, University of Chicago, Chicago, Illinois, USA

Accurate, timely human immunodeficiency virus (HIV) diagnosis is critical. Routine HIV screening program data were examined before and after reflex HIV type 1 RNA testing. Reflex testing facilitated confirmation of reactive HIV screening assays (as true or false positives) (odds ratio, 23.7 [95% confidence interval, 6.7–83.4]; $P < .0001$), improving detection of acute HIV and reducing unconfirmed discordant results.

Keywords. acute HIV infection; discordant results; HIV testing; reflex HIV-1 RNA.

Timely and accurate diagnosis of human immunodeficiency virus (HIV) is one of the critical pillars of the national Ending the HIV Epidemic (EHE) plan in the United States [1]. The Centers for Disease Control and Prevention's current algorithm for HIV diagnosis uses a fourth-generation screening assay, including both p24 antigen and antibody testing; a positive or indeterminate fourth-generation screening assay is followed by an HIV-1/HIV-2 antibody differentiation immunoassay [2, 3]. If the differentiation immunoassay is negative or indeterminate following a reactive screening assay (ie, discordant results), an HIV-1 and/or HIV-2 nucleic acid test for viral RNA is required to determine if the result represents a false-positive result or an acute HIV infection (AHI) [4]. At many healthcare organizations, HIV RNA assays require a second blood draw from the patient, which may result in loss of follow-up and inability to resolve discordant results [5].

To decrease unconfirmed cases and improve detection of AHI, experts have suggested updating the algorithm to perform the HIV RNA assay before the antibody differentiation immunoassay or to perform a reflex HIV RNA assay for all discordant results, though cost may be a barrier [6–9]. In this report, we examine unconfirmed and discordant results before and after implementation of a reflex HIV-1 RNA assay following all reactive fourth-generation screening tests at an academic medical center with a routine opt-out HIV screening program within an EHE priority jurisdiction. This program was started in 2011 and now performs >15 000 HIV tests per year, the majority in the emergency department [10]. We report on overall positive results, discordant results, and time for linkage/relinkage to care for new and previously diagnosed persons before and after the introduction of reflex HIV-1 RNA testing.

METHODS

Data from the routine HIV screening program at the University of Chicago Medicine (UCM) were collected from 14 May 2014 to 30 June 2021. During this period, UCM used a fourth- or fifth-generation screening assay followed by the antibody differentiation assay. We defined discordant test results as a reactive screening assay followed by a negative or indeterminate antibody differentiation assay; discordant results include confirmed false-positive results, unconfirmed results (patients who did not undergo HIV-1 RNA testing), and acute HIV cases. Acute HIV was determined by a reactive screening assay followed by a negative antibody differentiation assay and a positive HIV-1 RNA result. New HIV diagnoses also include acute HIV cases.

From 14 May 2014 to 27 September 2016, resolving discordant results required an additional blood draw to perform the HIV-1 RNA test. From 28 September 2016 to 30 June 2021, a reflex quantitative HIV-1 RNA test was performed following any reactive screening assay, including those with discordant results. Data from 1 October 2018 to 31 December 2018 were excluded due to an increase in false-positive results caused by an issue with the HIV screening assay reagent.

We examined the number of reactive screening assay results, confirmed true positives, discordant results, acute HIV cases, confirmed false positives, and discordant test results that remained unresolved. Median differences in time to linkage to care before and after reflex HIV-1 RNA testing were compared. We used χ^2 tests and odds ratios (ORs) with either 95%

Received 21 July 2023; editorial decision 06 December 2023; accepted 11 December 2023; published online 12 December 2023

Correspondence: Moira C. McNulty, MD, MSc, Section of Infectious Diseases and Global Health, University of Chicago, 5841 S Maryland Ave, MC 5065, Chicago, IL 60637 (moira.mcnulty@uchospitals.edu).

Open Forum Infectious Diseases®

https://doi.org/10.1093/ofid/ofad629

Table 1. Human Immunodeficiency Virus (HIV) Test Results Before and After Reflex HIV-1 RNA Testing, 14 May 2014 to 30 June 2021

Year	All Patients Tested	Reactive HIV Screening Assay[a]	Confirmed True-Positive Results[b]	New HIV Cases (Acute Cases[c])	Existing HIV Cases	Existing Cases Needing Linkage	Discordant Results (Acute Cases[c])	Confirmed False-Positive Results	Unconfirmed Reactive Screening Assay	Median Days to Linkage (No. Linked)
2014	6924	56	46	20 (0)	26	11	10 (0)	3	7	31 (7)
2015	10 710	72	65	27 (0)	38	9	7 (0)	2	5	20 (9)
2016	9611	62	57	32 (7)	25	9	12 (7)	4	1	14 (5)
Subtotal	27 245	190	168	79 (7)	89	29	29 (7)	9	13	20 (21)
HIV-1 RNA reflex begins 28 September 2016										
2016	3139	25	17	8 (0)	9	2	8 (0)	8	0	14 (4)
2017	15 450	240	90	35 (5)	55	11	155 (5)	149	1	9 (21)
2018[d]	12 791	211	77	27 (3)	50	12	137 (3)	133	1	9 (19)
2019	27 860	288	186	56 (8)	130	32	110 (8)	101	1	12 (29)
2020	28 610	354	276	49 (12)	227	51	90 (12)	78	0	7.5 (46)
2021[e]	18 096	253	207	33 (7)	174	48	53 (7)	46	0	8 (28)
Subtotal	105 946	1371	853	208 (35)	645	156	553 (35)	515	3	9 (147)
Total	133 191	1561	1021	287 (42)	734	185	582 (42)	524	16	20 (168)

Abbreviation: HIV, human immunodeficiency virus.

[a]The "Reactive HIV Screening Assay" column is the sum of the "Confirmed True-Positive Results" plus "Confirmed False-Positive Results" plus "Unconfirmed Reactive Screening Assay" columns.

[b]The "Confirmed True-Positive Results" column reflects new HIV cases (a subset of which are acute cases) plus existing HIV cases.

[c]Acute cases are included in both the "New HIV Cases" column as well as the "Discordant Results" column since they are part of both categories.

[d]Includes data from 1 January 2018 to 30 September 2018 only.

[e]Includes data from 1 January 2021 to 30 June 2021 only.

confidence intervals (CIs) or 2-sided *P* values of .05 being considered statistically significant. Analyses were performed in SAS version 9.4.

RESULTS

Prior to reflex HIV-1 RNA testing, from 14 May 2014 to 27 September 2016, there were a total of 190 unique patients with reactive HIV screening results; 168 were confirmed true-positive results (88.4%; 79 new diagnoses [7 of which were acute], 89 existing diagnoses). Of the 29 discordant results, 7 were acute cases (3.7%), 9 were false-positive results (4.7%), and 13 tests were unable to be confirmed (6.8%) (Table 1). These patients were either not able to be located or did not return for further testing at our institution. Of the reactive test results that were new, existing, or acute cases, 108 people required outreach to link or relinkage to HIV care.

After reflex HIV-1 RNA testing, from 28 September 2016 to 30 June 2021, there were 1371 reactive HIV screening assays in unique patients; 853 were confirmed true-positive results (62.2%; 208 new diagnoses [35 of which were acute], 645 existing diagnoses). Of 553 discordant results, there were 35 acute infections (6.3%), 515 confirmed false-positive results (37.5%), and 3 unconfirmed screening assay results (0.2%) (Table 1). Of reactive test results that were new, existing, or acute cases, 364 people required outreach to link or relinkage to HIV care.

With reflex HIV-1 RNA testing, reactive HIV screening assays were significantly more likely to be confirmed (either as true or false positives) compared to the period before reflex HIV-1 RNA testing was implemented (OR, 23.7 [95% CI, 6.7–83.4]; $P < .0001$). Similarly, newly diagnosed patients with HIV were linked to care significantly faster under reflex HIV-1 RNA testing (median difference, −7.6 days [95% CI, −12.7 to −2.6 days]; $P = .003$). Since implementing reflex HIV-1 RNA testing, acute cases have comprised 16.8% (35/208) of all new HIV diagnoses at UCM, nearly double the proportion detected before (8.9% [7/79]), although these results were not statistically significant (OR, 1.9 [95% CI, [.8–4.5]; $P = .14$). Including HIV-1 RNA testing significantly reduced the number of patients needing to be contacted regarding discordant test results or to initiate linkage/relinkage to HIV care (OR, 0.7 [95% CI, .6–.8]; $P = .001$).

DISCUSSION

At our institution, HIV-1 RNA reflex testing significantly decreased the number of discordant results and improved identification of AHI in a timely manner, while freeing resources to provide linkage to care and other important services. We were able to use reflex HIV-1 RNA testing to efficiently differentiate AHI from false-positive results, reflected in the number of acute cases detected after reflex HIV-1 testing began, despite no statistically significant difference. Accurately identifying people with AHI is crucial for preventing secondary transmission of HIV by providing linkage to care and initiation of antiretroviral therapy (ART) [11–13]. Given the large volume of

HIV screening tests performed, streamlined detection of true-positive HIV tests permitted growth of routine, opt-out HIV screening while allowing staff to prioritize contacting patients with true-positive results rather than tracking individuals with discordant results. It can be difficult to reach patients who require additional testing for HIV confirmation, and others may outright refuse to undergo further testing [14, 15]. In fact, the limited number of unconfirmed discordant cases following implementation of reflex HIV-1 RNA testing resulted from insufficient sample (6–10 mL blood sample required) to perform the HIV-1 RNA assay and subsequent inability to make patient contact. In the absence of HIV RNA assay results, use of the signal-to-cutoff ratio of the screening assay may also mitigate this problem, which can help distinguish between false-positive and acute (true-positive) results before confirmatory testing is available [15, 16].

By removing the need for a second blood draw in most circumstances, reflex HIV-1 RNA testing has supported rapid linkage and initiation of ART [17]. Rapid initiation of ART is a guidelines-supported intervention to shorten time to viral suppression and improve clinical outcomes [18–21]. The HIV screening algorithm plays a large role in ensuring that rapid ART initiation can occur [11, 12, 22]. Achieving rapid ART initiation can be facilitated by drawing a plasma sample that can be used for all steps of the diagnostic algorithm, thus avoiding delays caused by additional blood draws [7].

Finally, reflex HIV-1 RNA testing generated better detection not only of people who were newly diagnosed with HIV but also people with HIV (PWH) who had lapsed in care. In the emergency department setting, some PWH do not disclose their HIV status, resulting in retesting that can serve as an opportunity for relinkage. Reflex HIV-1 RNA testing has enabled staff to better determine who may be out of care and prioritize relinkage efforts among PWH with elevated viral loads. Certain laboratories such as the Mayo Clinic, LabCorp, and Quest Diagnostics have begun conducting reflex viral load testing only for people with discordant results [23–26]. In healthcare organizations like ours located within a high-HIV-prevalence area, there may be a benefit to conducting reflex HIV-1 RNA testing for *all* reactive screening assays due to the increased HIV burden and frequent transference of care between institutions. Similar settings may benefit from incorporating reflex HIV-1 RNA testing to avoid missed opportunities for diagnosing new and acute HIV infections, as well as ensuring PWH are linked or relinked to the appropriate care. This approach supports the Diagnose, Treat, and Respond pillars of the EHE initiative, thereby contributing to HIV elimination [27]. However, this approach may be cost-prohibitive; formal cost analyses accounting for benefits of early diagnosis and relinkage would contribute to our understanding of the tradeoffs of reflex testing for all reactive screening assays versus only for discordant test results, while the development of less expensive HIV-1 RNA viral load tests may allow for this approach to be adopted in the future with less concern for cost [7].

Limitations of this study include that it was conducted at a single urban academic medical center serving areas of high incidence and prevalence of HIV. Larger studies will need to be conducted to see if these results are consistent across different settings and patient populations. As a retrospective study, changes in clinical guidelines and best practices over time emphasizing the importance of rapid initiation of ART likely impacted the time to linkage to care, not reflex HIV-1 RNA testing alone. Additionally, it is possible that improvements in program and staff experience over time also contributed to reductions in time to linkage to care. Finally, this analysis excluded 3 months of data due to issues with testing reagents that caused many false-positive results; even so, there were more false-positive results in certain years likely due to changes in screening assays over time. It is unknown how the inclusion of those months would have affected the results.

CONCLUSIONS

Based on our results, we recommend that all high-incidence settings consider implementing reflex HIV-1 RNA testing to improve timely diagnosis of HIV, enable guideline-based rapid linkage and initiation of ART, and reduce resources required to resolve discordant results. Further work should include cost analysis to understand feasibility of such an approach across different healthcare settings.

Notes

Patient consent. Data for this study were collected as part of routine clinical care and for programmatic purposes through the routine HIV screening program. No informed consent was obtained. This study was determined to be exempt from institutional review board oversight at the University of Chicago.

Financial support. The Expanded HIV Testing and Linkage to Care Program is funded by the Health Resources and Services Administration (HRSA) through the Chicago Department of Public Health. Additional support is provided through the Gilead Sciences Frontlines of Communities in the United States (FOCUS) program. FOCUS funding supports HIV, hepatitis C virus, and hepatitis B virus screening and linkage to the first medical appointment after diagnosis; FOCUS funding does not support any activities beyond the first medical appointment and is agnostic to how FOCUS partners handle subsequent patient care and treatment. M. C. M. was supported by the National Institutes of Health (award number K23MH118969). K. A. S. is supported by the National Institutes of Health (award number K23AI166277).

Potential conflicts of interest. M. C. M. has served on an advisory board for Gilead Sciences, Inc. J. S. and D. P. report grant support from Gilead Sciences, Inc. All other authors report no potential conflicts.

References

1. US Department of Health and Human Services. America's HIV epidemic analysis dashboard. Available at: https://ahead.hiv.gov/. Accessed 15 June 2023.
2. Centers for Disease Control and Prevention. 2018 quick reference guide: recommended laboratory HIV testing algorithm for serum or plasma specimens. 2018. Available at: https://stacks.cdc.gov/view/cdc/50872. Accessed 15 June 2023.
3. Cornett JK, Kirn TJ. Laboratory diagnosis of HIV in adults: a review of current methods. Clin Infect Dis **2013**; 57:712–8.

4. Centers for Disease Control and Prevention. New CDC recommendations for HIV testing in laboratories: a step-by-step account of the approach. 2014. Available at: https://www.cdc.gov/nchhstp/newsroom/docs/2014/hiv-testing-labs-flowchart.pdf. Accessed 15 June 2023.
5. National HIV Curriculum. Available at: https://www.hiv.uw.edu/go/screening diagnosis/diagnostic-testing/core-concept/all#citations. Accessed 15 June 2023.
6. Sax PE. Time to fix the HIV testing algorithm—and here's how to do it. NEJM J Watch **2021**. Available at: https://blogs.jwatch.org/hiv-id-observations/index.php/time-to-fix-the-hiv-testing-algorithm-and-heres-how-to-doit/2021/02/15/. Accessed 15 June 2023.
7. Branson BM. HIV diagnostics: current recommendations and opportunities for improvement. Infect Dis Clin North Am **2019**; 33:611–28.
8. Pitasi MA, Patel SN, Wesolowski LG, et al. Performance of an alternative laboratory-based HIV diagnostic testing algorithm using HIV-1 RNA viral load. Sex Transm Dis **2020**; 47(5 Suppl 1):S18–25.
9. Duncan D, Duncan J, Kramer B, et al. An HIV diagnostic testing algorithm using the cobas HIV-1/HIV-2 qualitative assay for HIV type differentiation and confirmation. J Clin Microbiol **2021**; 59:e0303020.
10. Stanford KA, McNulty MC, Schmitt JR, et al. Incorporating HIV screening with COVID-19 testing in an urban emergency department during the pandemic. JAMA Intern Med **2021**; 181:1001–3.
11. Wang JS, Sim HJ, Park SJ, Park GR, Kang C, Chung YS. Effect of a confirmatory testing algorithm on early acute HIV diagnosis in Korea. Medicine (Baltimore) **2021**; 100:e25632.
12. Saz J, Dalmau-Bueno A, Meulbroek M, et al. Use of fourth-generation rapid combined antigen and antibody diagnostic tests for the detection of acute HIV infection in a community centre for men who have sex with men, between 2016 and 2019. PLoS One **2021**; 16:e0255065.
13. Amin SR, Patani H, Sukhija-Cohen AC, Rivera SM, Klausner JD. Acute human immunodeficiency testing and linkage to care: an evaluation of community-based 4th generation antigen/antibody testing. J AIDS HIV Res **2021**; 13:16–21.
14. Gu D, Mao Y, Tang Z, et al. Loss to follow-up from HIV screening to ART initiation in rural China. PLoS One **2016**; 11:e0164346.
15. Bailey AL, Anderson N. Risks of requiring a dedicated molecular specimen for HIV diagnosis and a potential strategy for mitigation. PLoS One **2020**; 15:e0237580.
16. Whitney E, Pitrak D, Beavis KG, et al. Utility of the signal-to-cutoff ratio and antigen index from fourth- and fifth-generation HIV-1/HIV-2 antigen/antibody assays for the diagnosis of acute HIV infection: applicability for real-time use for immediate initiation of antiretroviral therapy. J Clin Microbiol **2022**; 60:e0120422.
17. McNulty M, Schmitt J, Friedman E, et al. Implementing rapid initiation of antiretroviral therapy for acute HIV infection within a routine testing and linkage to care program in Chicago. J Int Assoc Provid AIDS Care **2020**; 19: 2325958220939754.
18. Bacon O, Chin J, Cohen SE, et al. Decreased time from human immunodeficiency virus diagnosis to care, antiretroviral therapy initiation, and virologic suppression during the citywide RAPID initiative in San Francisco. Clin Infect Dis **2021**; 73: e122–8.
19. Ford N, Migone C, Calmy A, et al. Benefits and risks of rapid initiation of antiretroviral therapy. AIDS **2018**; 32:17–23.
20. Saag MS, Gandhi RT, Hoy JF, et al. Antiretroviral drugs for treatment and prevention of HIV infection in adults: 2020 recommendations of the International Antiviral Society–USA panel. JAMA **2020**; 324:1651–69.
21. World Health Organization (WHO). Guidelines for managing advanced HIV disease and rapid initiation of antiretroviral therapy. Geneva, Switzerland: WHO; 2017.
22. Mashishi BR, Makatini Z, Adu-Gyamfi CG. The evolving HIV epidemic and its impact on the HIV testing algorithm: is it time to change the HIV testing algorithm in South Africa? J Clin Virol **2021**; 144:104990.
23. Labcorp. HIV p24 antigen/antibody with reflex to confirmation. 2023. Available at: https://www.labcorp.com/tests/083935/hiv-p24-antigen-antibody-with-reflex-to-confirmation. Accessed 15 June 2021.
24. Quest Diagnostics. HIV-1/2 antigen and antibodies fourth generation with reflexes. **2022**. Available at: https://testdirectory.questdiagnostics.com/test/test-detail/91431/hiv-1-2-antigen-and-antibodies-fourth-generation-with-reflexes?20cc=MASTER. Accessed 15 June 2021.
25. Mayo Clinic Laboratories. HIV-1 and HIV-2 antigen and antibody diagnostic evaluation, plasma. **2023**. Available at: https://www.mayocliniclabs.com/test-catalog/Overview/48392. Accessed 15 June 2023.
26. Mayo Clinic Laboratories. HIV testing algorithm (fourth-generation screening assay), including follow-up of reactive rapid serologic test results. 2023. Available at: https://www.mayocliniclabs.com/articles/resources/-/media/it-mmfiles/special%20instructions/8/d/4/hivtestingalgorithmfourthgenerationscreeningassayincludingfollowupofreactivehivrapidserologictestres.pdf. Accessed 15 June 2023.
27. Centers for Disease Control and Prevention. About the Ending the HIV Epidemic in the U.S. pillars. 2023. Available at: https://www.cdc.gov/endhiv/about-ehe/pillars.html. Accessed 15 June 2023.