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CORRESPONDENCE

Reducing time to new HIV diagnosis: time for change in the HIV diagnostic algorithm?

To the Editor,

In Australia, diagnosis of human immunodeficiency virus (HIV) infection has traditionally relied on a Western blot (WB) for confirmation. Prior to a recent update to the national laboratory case definition for HIV, laboratory confirmation of HIV diagnosis was fulfilled with a repeatedly reactive HIV antibody or combination antibody/antigen screening test followed by a positive WB, or positive p24 antigen confirmed by neutralisation on two separate specimens.[1] Although this approach maximises test specificity, it may be limited by prolonged time to HIV-specific IgG development for WB detection and need for repeated specimen collection for p24 assays. The Public Health Laboratory Network (PHLN) updated the HIV laboratory case definition in March 2022 to include nucleic acid amplification tests (NATs) as an alternative confirmatory assay to WB or p24 for HIV diagnosis.[2] The updated laboratory case definition also removed the requirement to collect a second specimen for p24 testing in adults and children aged over 18 months. These changes provide the opportunity for more rapid diagnosis, particularly in acute HIV infection. Here, we sought to assess time to laboratory-confirmed HIV diagnosis using the previous laboratory case definition, comparing time to confirmation using either WB or p24 assay. These results may inform development of an updated diagnostic algorithm to incorporate the recent changes to the national case definition.

To assess time to confirmed HIV diagnosis using the previous laboratory case definition, we reviewed cases of newly diagnosed HIV at the Royal Melbourne Hospital from 1 July 2017 to 31 May 2021. Results of the screening HIV immunoassay, WB, p24, HIV viral load and CD4 count were collected. Time to laboratory-confirmed HIV diagnosis was calculated from date of first sample collection for initial HIV testing to the date when case definition for HIV diagnosis was fulfilled. Where available, time from initial sample collection to antiretroviral therapy (ART) commencement was recorded. Differences between individuals diagnosed through WB and p24 assay were compared using Wilcoxon rank-sum using R statistical software (version 4.1.1, https://www.r-project.org/). p values <0.05 were regarded as significant. The study was approved by the Melbourne Health Human Research Ethics Committee as a quality assurance project (QA2021045).

Thirty-one cases of HIV were diagnosed during the study period. Twenty-five (81%) fulfilled case definition through positive WB, with median time to laboratory-confirmed HIV diagnosis of 5 days (range 2−10) (Table 1). Six cases with an indeterminate or negative WB fulfilled criteria by p24 assay with median time to confirmed HIV diagnosis of 10 days (range 5−20). Individuals diagnosed through p24 had significantly higher viral loads compared to individuals diagnosed through WB (median 1,273,358 vs 119,002 copies/mL, p=0.008), consistent with acute infection (Fiebig stages II−IV). Individuals diagnosed through WB had established infection: seven (28%) had advanced HIV with CD4 count $<0.05\times10^9$/L and 12 (48%) with CD4 count $<0.20\times10^9$/L on presentation. Data on ART commencement were available for 21 of the 31 cases. The 10 individuals with no data available were diagnosed through WB and received follow-up with external primary care providers. For the 21 individuals who commenced ART at our centre, therapy was commenced a median of 10 days post initial sample collection and was the same for individuals diagnosed through WB and p24. Median time to ART commencement for individuals diagnosed through WB with CD4 count $<0.05\times10^9$/L, $<0.20\times10^9$/L and $\geq0.20\times10^9$/L was 15, 12 and 9 days, respectively.

Our study highlights the limitation of the current diagnostic algorithm in confirming acute HIV infection using the previous case definition, with double the time required to confirm HIV diagnosis in patients with acute compared to established infection. High viral loads observed in acute infection have been associated with transmission risk of up to 26 times higher compared to chronic infection.[3] This has led to estimates of disproportionately higher transmission from individuals with acute infection in certain settings; in one study, 35% of new HIV diagnoses among casual sexual partners in men who have sex with men was attributed to transmission from individuals with primary HIV infection.[4] This highlights the importance of timely diagnosis of acute HIV infection in facilitating early counselling, treatment and contact tracing to prevent onward transmission in this group.

Although we observed longer time to laboratory-confirmed diagnosis in individuals with acute infection, we did not observe a difference in time to commencement of ART. This may partly be explained by the several cases of advanced HIV in those who were diagnosed through WB, represented by the low median CD4 count in this group of 0.16×10^9/L. In some of these individuals, ART was necessarily delayed in order to diagnose and manage opportunistic infections, such as cryptococcal meningitis, where ART commencement is deferred for up to 6 weeks after antifungals are started to avoid the immune reconstitution inflammatory syndrome. For individuals without clinical contraindication, ART should be commenced early. Accelerated or same day initiated ART at time of diagnosis has been associated with improved rates of viral suppression and retention in care.[5]

Newer approaches to confirmatory HIV testing have become available that could streamline the diagnostic process, particularly in acute infection. There are now several NATs approved for use by the Therapeutic Goods Administration (TGA) as confirmatory tests for HIV diagnosis in immunoassay-reactive individuals, including the Aptima HIV-1 Quant Dx assay (Hologic, Australia), cobas HIV-1/HIV-2 Qualitative Test (Roche Diagnostics, Australia), cobas HIV-1 assay for use on cobas 6800/8800 systems (Roche Diagnostics), and the Alinity m HIV-1 Kit (Abbott Australasia).[6] These are automated tests and have the advantage of being run either as single tests or in batches to reduce turnaround time. These assays have demonstrated specificity nearing 100% for HIV diagnosis, with a limit of detection of approximately 12−20 copies/mL.[7,8] The specificity of these tests are higher than that of quantitative HIV

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Table 1 Time to new HIV diagnosis, baseline tests and time to antiretroviral therapy commencement

	All new HIV diagnoses (n=31)	New HIV diagnosis by positive WB[a] (n=25)	New HIV diagnosis by p24 assay (n=6)	p value[b]
Days to HIV diagnosis[c]	5 (4−8)	5 (4−6)	10 (9−14)	0.004
HIV viral load, copies/mL	183,888 (101,766−822,415)	119,002 (85,704−512,466)	1,273,358 (436,986−4,139,515)	0.008
HIV viral load, log10	5.26 (5.01−5.92)	5.08 (4.93−5.71)	6.10 (5.64−6.62)	0.001
CD4 T cell count, $\times 10^9$/L	0.27 (0.06−0.51)	0.16 (0.04−0.34)	0.54 (0.51−0.64)	0.001
CD4 T cell, %	16.9 (6.4−24.3)	16.0 (4.2−21.5)	21.9 (13.8−28.3)	0.19
Days from sample collection to ART commencement	10 (7−18)	10 (7−19)	10 (8−12)	0.99

Data are shown as median and interquartile range.
ART, antiretroviral therapy; WB, western blot.
[a] Positive western blot defined as detection of 2 ENV bands (gp160 and/or gp41 and gp120) with GAG (p17, p24, p55) or POL (p31, p51, p66).
[b] Comparison of individuals diagnosed with HIV by WB versus p24 assay using Wilcoxon rank-sum.
[c] Diagnosis as per Australian Public Health Laboratory Network 2015.[1]

viral load assays, with several cases of false-positives previously reported using quantitative assays for diagnosis.[9] NATs also have the potential advantage of detecting very early HIV infection, prior to antibody or p24 detection. Using HIV seroconversion panels, HIV-1 could be detected by the Aptima assay 6 days before the p24 antigen,[7] and by the cobas assay 19 days earlier than the Bio-Rad Geenius HIV 1/2 Confirmatory Assay.[8] One further advantage of the cobas HIV-1/HIV-2 qualitative assay over WB is differentiating HIV-1 and HIV-2, although prevalence of HIV-2 in Australia remains rare.

In the United States, NATs are currently only recommended to resolve discrepant results from positive screening immunoassays and negative or indeterminant confirmatory HIV-1/HIV-2 antibody differentiation immunoassay results, as can occur in acute infection. In the United Kingdom, NATs have been listed as an alternative to immunoassays for confirmatory HIV diagnosis, although this is not yet part of standard testing. The cost benefit of this approach has not yet been formally assessed; however, consideration should be given to its potential impact on total testing numbers from reduced indeterminate confirmatory test results, as well as benefits of faster turnaround time, early diagnosis, and reduced transmission.

In Australia, the current National Pathology Accreditation Advisory Council (NPAAC) guidelines for laboratory testing of HIV stipulate WB be used for confirmatory testing and that NATs cannot currently be used for laboratory confirmation of HIV.[10] The NPAAC guidelines were last updated in 2013 and developed prior to TGA approval of qualitative HIV NATs for diagnosis. In order for clinical laboratories to implement HIV NAT use in HIV diagnosis, these guidelines will require revision to align with the updated PHLN case definition.

One limitation when using NATs for confirmatory testing is the potential for false negative results in individuals with undetectable viral load,[11] as can occur in elite controllers or individuals on ART that has not been disclosed. This includes individuals who have continued pre-exposure prophylaxis (PrEP) following HIV acquisition, where viral loads have been shown to be lower during seroconversion compared to individuals not on PrEP, with 11% of individuals demonstrating an undetectable viral load.[12] A large study examining the use of HIV NAT as a second-line assay for confirmation of HIV diagnosis was recently conducted by Duncan *et al.*[11] This study described the performance of an alternative diagnostic algorithm using the cobas HIV-1/HIV-2 qualitative NAT as the second-line assay for HIV confirmation compared to the Geenius HIV 1/2 supplemental immunoassay. Negative percent agreements for HIV-1 and HIV-2 was >99% for HIV-1 and HIV-2 for all groups. Positive percent agreement was 100% in the known HIV-1-positive group, however positive percent agreement was lower than expected in a number of groups (58%, 77.7%, 66.7%, for HIV-1 high risk, HIV-2 high risk and HIV low risk populations, respectively). The primary reason for this low positive percent agreement was positive serology results with undetectable HIV RNA. This may have been due to undisclosed ART use such as PrEP, elite controllers as described above, or due to false-positive serology results. This study demonstrates that additional confirmatory testing with traditional methods such as WB, HIV-1/HIV-2 antibody differentiation immunoassay or HIV proviral DNA is likely to be required as a third test in a HIV NAT-based algorithm for fourth-generation immunoassay screen-positive/NAT-negative specimens.

The updated national case definition now provides opportunity for NATs to be incorporated into the HIV diagnostic algorithm, however the implementation of significant changes to HIV testing by laboratories is currently limited by discordant NPAAC standards. Incorporation of these newer tests into a HIV diagnostic algorithm could significantly reduce time to confirmation of HIV diagnosis while importantly retaining high test specificity.

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