The potential complexity and need for caution when interpreting atypical human immunodeficiency virus reactivity in blood donors

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Introduction

Since the mid-1980s, blood centres worldwide have screened donors for antibodies to human immunodeficiency virus (anti-HIV) and, more recently, many have also implemented screening for HIV by nucleic acid tests (NAT)[1,2]. Reactivity on anti-HIV screening immunoassays is typically confirmed by immunoblot testing which can, however, generate indeterminate results. In low-risk voluntary blood donors, such indeterminate results usually represent non-specific reactivity[3-6]. However, this result profile can sometimes occur in HIV-infected individuals undergoing antiretroviral therapy[7], and HIV elite controllers who appear to be able to control HIV replication without antiretroviral therapy[8,9]. In this report we present a case study which highlights the potential complexity and need for caution when interpreting HIV reactivity in blood donors.

Case report

During the donor follow-up period (March, 2012 to August, 2013), the Australian Red Cross Blood Service (Blood Service) screened all donations for anti-HIV-1/2 using the Prism HIV O Plus chemiluminescent immunoassay (Abbott Diagnostics, Delkenheim, Germany) and donations that tested repeatedly reactive were further tested on the Genscreen Ultra HIV Ag-Ab enzyme immunoassay (EIA) (Bio-Rad, Redmond, WA, USA). Donations that tested reactive on both immunoassays were referred to the Victorian Infectious Diseases Reference Laboratory (VIDRL) for anti-HIV western blot testing (MP Diagnostics HIV Blot 2.2, Genelabs, Singapore) as well as additional HIV serological testing and NAT. In Australia, anti-HIV western blot positivity is defined as reactivity to at least one glycoprotein band and at least three non-envelope protein bands, western blot negativity is defined as no reactivity to any HIV-specific proteins, while indeterminate status is defined as reactivity to one or more HIV-specific protein bands without meeting the criteria for positivity[10]. For third-generation anti-HIV immunoassays, the window period from infectiousness to antibody detection has been estimated to be 22 (range, 6 to 38) days[11]. The Genscreen Ultra HIV Ag-Ab EIA detects HIV p24 antigen and the window period for HIV p24 antigen detection has been estimated to be 15.0 (range, 13.3-16.7) days[12].

In addition to anti-HIV screening, all donations were screened for HIV-1 RNA, hepatitis C virus (HCV) RNA and hepatitis B virus (HBV) DNA using the Procleix Ultrio HIV-1/HCV/HBV multiplex assay (Ultrio) on the Procleix Tigris automated platform (Gen-Probe/Novartis Diagnostics, San Diego/Emeryville, CA, USA). Samples reactive on the Ultrio assay were "discriminated" to identify the specific virus using Procleix HIV-1, HCV and HBV discriminatory assays. HIV-1 RNA testing was also performed at the VIDRL using either the Abbott Real Time HIV-1 reverse transcriptase polymerase chain reaction assay (Abbott Diagnostics, Delkenheim, Germany) or Roche Ampliprep/Taqman HIV-1 test version 2.0 (Roche Diagnostics, Indianapolis, IN, USA). The HIV-1 RNA 95% limits of detection for the Ultrio and HIV-1 discriminatory assays are given by the manufacturer as 28.79 (95% fiducial limits, 25.85-32.68) IU/mL and 32.15 (95% fiducial limits, 28.74-36.70) IU/mL, respectively[13]. Blood Service modelling based on the method of Busch *et al.*[12] estimated that the infectious window period for the Ultrio assay is 5.6 (range, 5.0 to 6.8) days. The Procleix Ultrio assay is a qualitative *in vitro* NAT for use on the Procleix Tigris System to screen for HIV-1 RNA, HCV RNA and HBV DNA. The Ultrio HIV Discriminatory (dHIV) assay is specific for the detection of HIV-1 RNA. The Ultrio assay detects HIV-1 groups M (subgroups A-H), N and O[13]. The Roche assay is a dual target assay (Gag and LTR) detecting HIV groups M (subgroups A-H) and O with detection limits of 20 copies/mL to 10,000,000 copies/mL[14]. The Abbott assay is a single target assay (Integrase), detecting HIV groups M (subgroups A-H), N and O with detection limits of 150 copies/mL to 10,000,000 copies/mL for the 0.2 mL protocol[15]. The proviral DNA assay used at the VIDRL is a single target (LTR) in-house real-time assay performed on the ABI 7500 and samples are tested in duplicate along with an internal control.

The donor was male, 34 years of age at the index donation (17 March 2012) and a first-time donor. Follow-up testing was performed by the Blood Service and the VIDRL on samples taken on 12 April, 10 May, 12 June and 21 June 2012. In addition, samples were taken on 16 August

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and 29 November 2012, and 12 March and 9 August 2013 for testing at the VIDRL. The donor was interviewed by a medical officer/counsellor and no specific risk factors for HIV infection were identified. The donor declared that he was not undergoing antiretroviral treatment.

Results for the index donation and four follow-up samples are summarised in Table I. All were strongly reactive on the Prism HIV O plus and Genscreen assays at the Blood Service, and on the Murex HIV EIA at the VIDRL. All samples were indeterminate according to western blotting. The index donation was non-reactive on the Ultrio assay and not tested on the dHIV assay, while the first follow-up sample was non-reactive on both the Ultrio and dHIV assays. However, the second follow-up sample, although non-reactive on the Ultrio assay, was reactive on the dHIV assay. The follow-up sample taken on 21 June 2012 was tested in 20 replicates on the dHIV assay and two of 20 were reactive. In contrast, six and nine replicates of the follow-up samples taken on 12 and 21 June 2012, respectively, and tested at the VIDRL on the Roche Taqman assay were all non-reactive.

The next three follow-up samples (16/08/12, 29/11/12 and 12/03/13) were tested by the VIDRL. All three remained reactive on the Murex and Genscreen Ultra EIAs were western blot indeterminate and HIV-1 RNA was not detected by either the Roche Ampliprep/Taqman or Abbott Real Time HIV-1 reverse transcriptase polymerase chain reaction assays. In addition HIV-1 proviral DNA was not detected in the buffy coat from samples collected on 12/06/12, 21/06/12 and 12/03/13. A final sample was taken on 9 August 2013 and remained indeterminate by western blotting with HIV RNA not detected.

Discussion

A sequential anti-HIV immunoassay screening strategy is applied at the Blood Service whereby donor samples that test reactive on the primary anti-HIV immunoassay, and non-reactive by NAT, are only referred for western blot testing if reactive on the secondary anti-HIV immunoassay[16]. As a consequence, only a relatively small number of donors are tested by anti-HIV western blot and they are typically negative or indeterminate. Upon follow-up, they do not progress to become western blot-positive and remain non-reactive by HIV NAT. Between July 2010 and December 2014, approximately 5.9 million donations were tested by the Blood Service, of which seven gave anti-HIV indeterminate results by western blot and all were non-reactive on Ultrio or Ultrio Plus assays. However, the profile of this donor's results was unusual for two reasons. Firstly, the Prism HIV O Plus assay sample-to-cutoff (s/co) ratios for samples from this donor varied between 60.9 and 83.3 and, in our donor population, s/co ratios this high had not previously been observed without concomitant HIV NAT reactivity. In a previous study of our donor population it was found that 97.4% of donors with non-specific reactivity on the Prism assay had s/co ratios <10.0 and none had s/c ratios >60.0[17]. In contrast, the Prism assay s/co ratios for this donor were within the range (40.0 to 200.0) observed for most HIV-positive donors (western blot-positive/HIV-1 RNA-positive).

Table I - Summary of the HIV serology and NAT results for the index donation and four subsequent follow-up samples from the case study donor. Results are included for testing at both the Australian Red Cross Blood Service (Blood Service) and the Victorian Infectious Diseases Reference Laboratory (VIDRL).

Date	Blood Service results				Victorian Infectious Diseases Reference Laboratory (VIDRL) results			
	Serology		*NAT*		*Murex 1.2 O HIV EIA*	*Western blot*[5]	*Abbott Real Time*	*Roche*[6]
	Prism HIV O Plus s/co ratios[1]	*Genscreen HIV Ultra s/co ratios*[2]	*Ultrio*[3]	*dHIV*[4]				
17/03/12 (index donation)	60.9 69.0, 67.2	7.5, 7.5	NR	NT	REA	p24++, gp160++	ND	NT
12/04/12	77.5 65.8, 65.5	11.7, 11.7	NR	NR	REA	p24++, gp 120+, gp160++	NT	ND
10/05/12	65.5 66.4, 66.7	13.0, 13.1	NT	REA	REA	p24++, gp 120+, gp160++	ND	NT
12/06/12	61.1 70.3, 65.3	13.2, 13.2	NR	NR	REA	p24++, gp 120+, gp160++	NT	ND[7]
21/06/12	70.3 83.3, 71.1	13.1, 12.2	NR	REA[8]	NT	NT	NT	ND[9]

[1]s/co ratio: sample to cutoff ratio; s/co ratio ≥1.0 = REA; s/co ratios for initial test and duplicate repeat tests; [2]s/co ratio: sample to cutoff ratio; s/co ratio ≥1.0 = REA; samples tested in duplicate; [3]Ultrio: Procleix Ultrio multiplex assay; [4]dHIV: Procleix HIV-1 discriminatory assay; [5]MP Diagnostics HIV Blot 2.2; [6]Roche Ampliprep/Taqman HIV-1 Test version 2.0; [7]six of six replicates were non-reactive; [8]two of 20 replicates were reactive; [9]nine of nine replicates were non-reactive.
NAT: nucleic acid testing; HIV: human immunodeficiency virus; NR: non-reactive; NT: not tested; REA: reactive; ND: not detected.

Secondly, two samples from this donor tested reactive on the dHIV assay, one sample was reactive when tested once while a second sample tested reactive in two of 20 replicates. This combination of high s/co ratios on the Prism assay with intermittent NAT reactivity suggested the possibility that this donor may be a "very elite" HIV controller, a recently reported phenomenon characterised by even lower HIV RNA levels than those found in typical HIV elite controllers[9].

However, over the 17-month follow-up period, while all samples remained strongly reactive on the anti-HIV immunoassays, there was no progression to a western blot-positive result. HIV RNA was not detected in any of the donor's samples tested at VIDRL on the Abbot Real Time or Roche Taqman assays, despite replicate testing on the Roche assay on two occasions. In addition, three samples were tested for proviral DNA and all three were negative. After the 17-month follow-up period, which included both laboratory testing and clinical assessment, the donor's results were assessed as not consistent with HIV infection. Given this assessment, the discordant results for the Ultrio Plus and dHIV assays could be due to contamination or represent false positive results but we were unable to distinguish between these two explanations.

It should be noted that this particular case did not represent a risk to blood safety. In Australia, donations that test repeatedly reactive on a serology screening assay for anti-HIV, anti-HCV or HBsAg are discarded regardless of confirmatory serology testing and NAT results.

In summary, the HIV test results for this donor are unusual for our donor population and highlight the potential complexity of interpreting atypical HIV serological and NAT results in blood donors. Given that our donors are typically at low risk of HIV infection, this case study also highlights the need for appropriate follow-up testing when a donor's HIV status cannot be definitively determined on the index donation, and the need for caution when counselling such donors regarding their HIV status.

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Authorship contributions

PK was primarily responsible for the initial draft. All Authors contributed to draft development and critical analysis, as well as collation and interpretation of results.

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