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TRANSPLANT INFECTIOUS DISEASE

ORIGINAL ARTICLE

Revised nucleic acid test window periods: Applications and limitations in organ donation practice

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Abstract

Background: Nucleic acid test window periods for HIV, HCV, and HBV facilitate estimation of the residual risk of unexpected disease transmission and assist clinicians in determining the timeframe in which a recently acquired infection is at risk of nondetection.

Objectives: Firstly, to provide revised estimates of the NAT window periods based on a currently used triplex NAT assay. Secondly, to examine their validity in organ donation and transplantation practice.

Method: Estimates were based on the Procleix Ultrio Elite Assay (Grifols Diagnostic Solutions Inc. California, USA). The manufacturer's X50 and X95 limits of detection (LOD) were utilised. Viral doubling times of 0.85, 0.45, and 2.56 days and conversion factors for IU per ml to copies per mL of 0.6, 3.4, and 5 were assumed for HIV, HCV, and HBV respectively. Window periods were derived from the X50 and X95 LODs, based on a range of potential inoculum volumes.

Results: Calculated X50 window periods were 5.1 (4.5–5.8), 2.7 (2.4–2.9), and 16.6 (14.2–19.1) days for HIV, HCV, and HBV respectively.

Calculated X50 window periods, based on whole body plasma volume, were 11.8 (10.3–13.3), 6.2 (5.6–6.8) and 36.7 (31.3–42.1) days respectively.

Conclusion: X50 NAT window periods were significantly shorter for HBV and HCV and sit at the lower range of previously published estimates for HIV . Current modeling assumptions may not account for all unexpected transmission events and may no longer be suitable for application to organ donation and transplantation.

1 | INTRODUCTION

In 2019, the Transplant Society of Australia and New Zealand (TSANZ) recommended all patients who commenced work-up for deceased organ donation undertake routine nucleic acid test (NAT) screening for human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV).[1] Donor infection during the NAT window period may result in transmission of disease to transplant recipients and is the most common cause of unexpected donor derived infection.[2]

Determination of a test's window period length is integral to estimating the risk of unexpected transmission of blood borne viruses. Window periods assist in the quantitative estimation of the risk of unexpected disease transmission,[3,4] and guide clinicians in determining the interval prior to blood draw where a newly acquired infection may not be reliably detected.[1,5]

List of Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; LOD, limit of detection; NAT, nucleic acid test; SANBS, South African National Blood Service; TSANZ, Transplantation Society of Australia and New Zealand.

TABLE 1 Published nucleic acid test (NAT) window period estimates for human immunodeficiency virus (HIV), hepatitis C virus (HCV), and hepatitis B virus (HBV) assays.

Virus	Window period (days)	LOD used	Primary reference	Cited by
HIV	5.6	X50	[8]	AST, CST, ASTS, TSANZ[1,10]
	7	NS	[11]	AST, CST, ASTS, TSANZ[1,10]
	6	X50	[12]	Australian Red Cross Lifeblood
	4.0	X50	[7]	SANBS (Procleix Elite)
	3.7	X50	[7]	SANBS (COBAS MPX)
	3.3	X50	[9]	
HCV	4.9	X50	[8]	AST, CST, ASTS, TSANZ[1,10]
	7	NS	[11]	AST, CST, ASTS, TSANZ[1,10]
	3	X50	[12]	Australian Red Cross Lifeblood
	2.8	X50	[7]	SANBS (COBAS MPX)
	2.5	X50	[7]	SANBS (Procleix Elite)
	1.5	X50	[9]	
HBV	20.6	X50	[13]	AST, CST, ASTS, TSANZ[1,10]
	18.1	X50	[7]	SANBS (Procleix Elite)
	19.5	X50	[14]	AST, CST, ASTS, TSANZ[1,10]
	16	X50	[12]	Australian Red Cross Lifeblood
	14.4	X50	[7]	SANBS (COBAS MPX)
	10.3	X50	[9]	

Abbreviations: AST, American Society of Transplantation; ASTS, American Society of Transplant Surgeons; CST, Canadian Society of Transplantation; HIV, human immunodeficiency virus; HCV, hepatitis C virus; HBV, hepatitis B virus; LOD, Limit of detection; LOD, Limit of detection; NS, Not stated within paper; SANBS, South African National Blood Service; TSANZ, Transplant Society of Australian and New Zealand; X50, 50% Sensitivity.

Window periods are not specified by the manufacturer in their product information/package insert.[6] Rather, window periods are estimated based on (1) manufacturer designated assay sensitivity characteristics, (2) assumptions around the minimum infective dose and (3) early infection phase viral growth characteristics.[7,8] Methods for the estimation of NAT window periods have been driven by their applications in transfusion medicine, and the selection of specific thresholds for the latter two assumptions have been historically based on their applications in this field.

Window periods are predominantly used in transfusion medicine to estimate the residual risk of an unexpected transfusion transmitted infection. To date, published window period estimates, regardless of assay or platform, use a viral load associated with a 50% probability of assay reactivity (X50 Limit of Detection [X50 LOD]), and a minimum infective dose, which is typically 0.05 viral copies per mL[8,9].

The X50 LOD sensitivity threshold is used as a convenient "mathematical shortcut" that circumvents calculation of the total area under the sensitivity curve when estimating the probability of a false nonreactive result.[9] The minimum infective dose is derived from the plasma viral load equivalent to a single virion in the residual 20 mL of plasma found in an average unit of packed red blood cells.[8]

With newer, improved assays, current estimates are now significantly shorter than consensus values that have been widely adopted by the organ donation and transplant sectors (Table 1).

There are reasons to question the external validity of these assumptions for organ donation and transplantation medicine.

Firstly, current window periods may systematically underestimate the interval prior to blood draw, where increased risk behaviors could result in undetected infection. Understanding this interval is important to clinicians assessing risk in organ donors with a history of recent abstinence from increased risk behaviors, or where duration of final hospital admission has exceeded the presumptive window period for a given virus. Use of currently published window periods in these circumstances may be inappropriate as by definition, there is still a 50% chance of not detecting an infection acquired *immediately* outside the window period.

Secondly, assumptions about the donor plasma viral load associated with a minimum infective dose in a transplanted organ are unlikely to be valid for a variety of reasons. Transplanted organs have a significant variation in in vivo plasma volumes, both between ages and genders, and most notably between transplanted organs (e.g., kidney vs. liver).[15] Surgical flushing and perfusion practices additionally modulate these residual plasma volumes. Finally, transplantable organs have additional extravascular compartments, for which blood borne viruses have specific tropisms. The importance of these extravascular compartments in donor derived transmission has not been quantified, however as an example, reactivation of intracellular HBV, from NAT negative, serologically positive liver donors is well described.[16]

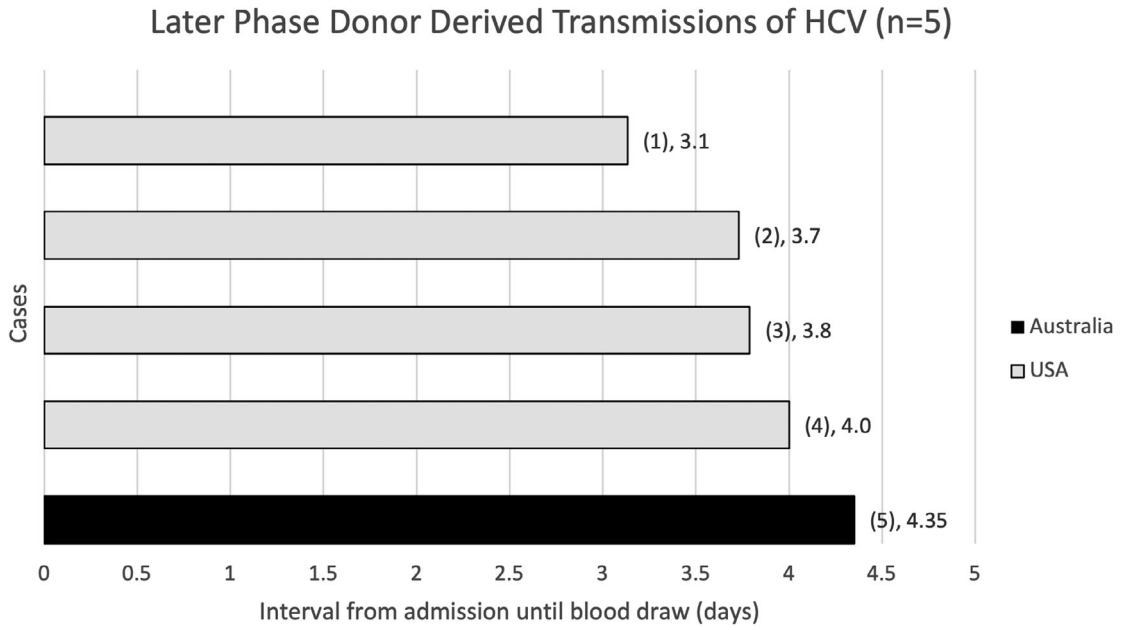

FIGURE 1 Cases of later phase donor derived transmissions of HCV[†].
(1) History of illicit drug use, HCV Ab-ve, NAT-ve on screening, USA.(2) History of intravenous drug use, Ab+ve, NAT-ve on screening, USA.(3) History of sex with an increased risk sexual partner, Ab-ve, NAT-ve on screening, USA.(4) History of intravenous drug use. Ab-ve, NAT-ve on screening, USA.(5) History of intravenous drug use. Ab-ve, NAT-ve on screening, Australia.
[†]Later phase defined as >3 days posthospital admission.
Data derived from Organ Procurement and Transplantation Network, US Department of Health and Human Services 2013–2017),[17] and the Australian Vigilance and Surveillance System for Organ Donation for Transplantation, Australian Organ and Tissue Authority.[5]

Concerns about the external validity of these assumptions has been reinforced by several cases of unexpected HCV transmission, which appear to have been acquired by donors, outside the window periods associated with individual donor samples tested on modern assays (Figure 1).

A case of unexpected donor derived HCV infection to multiple recipients has recently been described in Australia. In this case, the donor sustained an out-of-hospital cardiac arrest as a complication of injecting drug use and subsequently died in hospital. A non-hemodiluted blood sample was drawn from the patient 4.35 days post prehospital cardiac arrest. The sample was screened on the COBAS 4800 platform using the Roche COBAS HCV TEST (Roche Molecular Diagnostics, California) and returned a non-reactive (negative) result.[5] Based on reported assay sensitivities and current window period estimation methods, infections acquired greater than 3.8 days prior to blood draw would be expected to be detected by this assay (see Supplementary Material).

These cases raise questions about the applicability of current window period estimates to contemporary organ donation and transplantation practice.

In this paper, we aim to calculate the NAT window periods based on a contemporaneous triplex screening assay in common use. Additionally, we will model the impact of several underlying assumptions for this window period estimate.

2 | METHODS

2.1 | Setting and NAT assay

In addition to testing all blood donations collected in Australia for a number of infectious disease markers, Australian Red Cross Lifeblood (Lifeblood) also screens organ and tissue donor specimens for various infectious diseases. In 2021 Lifeblood implemented the Procleix Ultrio Elite Assay (Grifols Diagnostic Solutions Inc, Emeryville, California) as the new screening assay for HIV, HBV, and HCV.

The Ultrio Elite assay is a qualitative in vitro NAT licensed by the Australian Therapeutic Goods Administration for the detection of HIV-1 and HIV-2 RNA, HCV RNA, and HBV DNA in plasma or serum specimens.[18] Approved applications include blood donor screening, and individual donor screening of potential organ and tissue donors, either prior to or after cessation of circulation.[18]

The window period estimates calculated in this paper are specific to this assay.

2.2 | Calculation of NAT window periods

Window period lengths were calculated using the principles described in Busch et al.,[8] which is applied to one of the methods used to estimate

TABLE 2 Predicted limits of detection for Ultrio Elite 50% and 95% detection rates[†].

	Limit of detection	
Virus	X50 (95% CI)IU/mL	X95 (95% CI)IU/mL
HIV-1	5.4 (4.5 to 6.1)	18.0 (15.0 to 23.5)
HCV	0.9 (0.8 to 1.0)	3.0 (2.5 to 3.9)
HBV	0.9 (0.8 to 1.1)	4.3 (3.8 to 5.0)

[†]Manufacturer specified values.

the risk of transfusion transmissible infections in Australia.[19,20] In brief, the infectious window period is defined as the interval between the time a donor's viral load reaches the minimum level to render the donated "product" infective, and the time the viral load has reached the threshold of detection by the assay (see Equation 1).

$$WP = \frac{\log_{10}(v \cdot s \cdot x)}{\log_{10}(2)} \cdot t, \quad (1)$$

where

WP = window period

v = inoculum volume (mL)

s = pool size (n =)

x = detection limit (copies per mL)

t = virus doubling time (days)

Window period 95% confidence intervals were estimated using Monte Carlo simulation performed within Stata/IC 15.1 for Mac, StataCorp, College Station, TX. The simulation incorporated previously published confidence intervals for viral doubling times and the X50 LOD of the assay. Mean and 2.5 and 97.5 centiles were calculated on a set of 100, with 1000 iterations.

2.3 | Assumptions

The model assumes viral doubling times were 0.85 (CI: 0.76–0.97), 0.45 (CI: 0.41–0.50), and 2.56 (CI: 2.24–2.97) days for HIV, HCV, and HBV respectively.[21,22] The pool size value was 1, as individual donor NAT screening is routinely undertaken for organ, tissue, and blood donors in Australia.[18]

The minimum infective dose was conservatively assumed to be a single virion per inoculum volume. Manufacturer specified detection limits, as determined by probit analysis, were utilised (Table 2). To convert IU per mL to copies per mL the previously described conversion factors of 0.6,[23,24] 3.4,[25] and 5[23] for HIV, HCV, and HBV respectively were used.[26]

2.4 | Modeling the effect of inoculum volume

Modeling of window period estimates has been traditionally conducted on an inoculum volume of 20 mL.[9] In addition to this reference plasma volume, we modeled a range of inoculum volumes based on plasma volumes in various transplantable organs. Reference organ plasma volumes are based on previously published nuclear medicine studies of organ whole blood volume, assuming a plasma volume percentage of whole blood of 60%[27] and are based on average adult organ volumes.[15] Total plasma body volume is derived from the average value between male and female reference volumes. Fresh frozen plasma volumes are based on the average volume for a single Australian unit of fresh frozen plasma.[28]

TABLE 3 Estimated nucleic acid test (NAT) window periods for human immunodeficiency virus (HIV), hepatitis C virus (HCV), and hepatitis B virus (HBV) assays[†].

	Window Period Days (95% CI)		
Virus	X50 derived	X95 derived	TSANZ reference values[5]
HIV	5.1 (4.5–5.8)	6.6 (5.9–7.6)	5–6
HCV	2.7 (2.4–2.9)	3.5 (3.1–3.8)	3–5
HBV	16.6 (14.2–19.1)	22.4 (19.1–25.7)	20–22

Abbreviation: TSANZ, Transplantation Society of Australia and New Zealand.

[†]Based on 20 mL plasma inoculum volume.

2.5 | Determining cumulative risk of a false nonreactive result

We calculated the cumulative probability of a false nonreactive result, assuming risk of disease exposure is fixed and constant before blood draw, assuming exponential early viral growth and discretising calculations at 0.1 day intervals. The assay's sensitivity curve was assumed to follow the lognormal distribution, with characteristics informed by the manufacturer's X50 and X95 LOD thresholds. Expanded methods and calculations can be found in the Supplementary Material.

3 | RESULTS

The estimated window period for NAT screening of HIV, HCV, and HBV infections, utilizing the Procleix Ultrio Elite assay are summarized in Table 3. The results are based on a standardized plasma inoculum volume of 20 mL.

For HIV, the 95% confidence intervals for the estimated X50 window period sit within the currently recommended TSANZ window period range. In contrast, the estimated X50 window periods for HBV and HCV are significantly shorter than the current reference range.[1]

Estimated X95 window periods for all three viruses are significantly longer than the corresponding X50 estimates. The upper 95% confidence limit for the HIV and HBV X95 estimates are higher than the current reference ranges.[1]

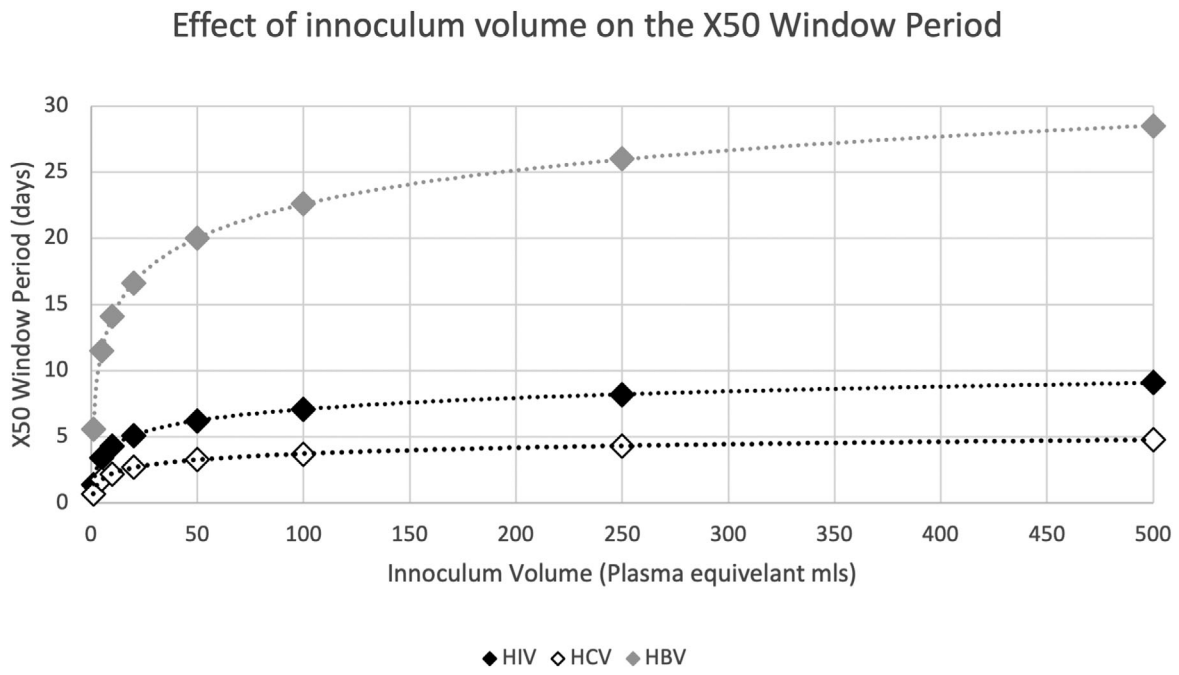

FIGURE 2 Effect of inoculum volume on window period estimates.

3.1 | Effect of inoculum volume

The effect on window periods of differing inoculum volumes has been modelled. The logarithmic impact of inoculum volume can be visualised in Figure 2 (See also Supplementary Material).

Whilst heart and single kidneys have an approximately equivalent plasma volume to a unit of packed red blood cells, lung and liver have significantly higher in-vivo plasma volumes. Un-remediated, these volumes would be expected to be associated with a relative prolongation of the NAT window periods for these donor organs (Table 4).

If we conservatively base the inoculum volume on the donor's whole plasma volume (4600 mL), we estimate the X50 window periods to be 36.7 (31.3–42.1), 6.2 (5.6–6.8) and 11.8 (10.3–13.3) days, for HBV, HCV, and HIV respectively. We estimate that 95% of the risk of a false nonreactive result occurs within 36, 6, and 11 days postinfective exposure for HBV, HCV, and HIV, respectively.

TABLE 4 Window period estimates for specific blood products and transplantable organs.

	Plasma Volume (mL)	X50 window period (days)			X95 window period (days)		
		HIV	HCV	HBV	HIV	HCV	HBV
PRBC	20	5.1	2.7	16.6	6.6	3.5	22.4
FFP[†]	278	8.3	4.4	26.3	9.8	5.2	32.1
Heart[‡]	28	5.5	2.9	17.8	7.0	3.7	23.6
Kidney[‡]	28	5.5	2.9	17.8	7.0	3.7	23.6
Liver[‡]	276	8.3	4.4	26.3	9.8	5.2	32.1
Lung[‡]	345	8.6	4.5	27.1	10.1	5.3	32.9

Abbreviations: FFP, fresh frozen plasma; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; PRBC, packed red blood cells.

[†]Based on average FFP bag volume.[25]

[‡]Based on Nuclear Medicine estimates of adult whole blood volume[15] averaged between sexes, assuming a plasma volume of 60% of whole blood volume.[25]

4 | DISCUSSION

This is the first published report of window period estimates for the Procleix Ultrio Elite triplex NAT assay for HIV, HCV, and HBV based on the manufacturers reported assay sensitivities, using both X50 and X95 limits of detection.

Our model produced updated X50 window period estimates that may be of use in estimating the residual risk of infection from the transfusion of packed red blood cells. Our estimates are similar to previously published data on the Procleix Ultrio Elite assay from the South African National Blood Service (SANBS).[7] We report slightly longer window periods for HIV (5.1 vs. 4.0 days) and HCV (2.7 vs. 2.5 days), and a slightly shorter period for HBV (16.6 vs. 18.1 days). The SANBS study independently derived LODs from local and international reference panels. Slight variations between study results, may in part be due to differences in the limits of detection used. This was most pronounced for the 95% LOD for HBV (21.5 copies/mL based on manufacturer's [Grifols] data vs. 47.9 copies/mL [SANBS]).

Since the publication of consensus window periods for NAT testing of organ donors, revised window periods for newer assays have been established. We present X50/20 mL estimates that are significantly

shorter for HBV and those that sit at the shorter end of the national consensus window period range for HIV and HCV.

We present a local case of unexpected donor derived HCV infection and demonstrate that the nonreactive result has most likely occurred despite the infection having been acquired outside the window period. This case further adds to a series of later phase unexpected donor derived infections screened using individual donor NAT, which would normally have been expected to be detected by modern systems. Together, these cases invite reflection on the currently incorporated assumptions used in the estimation of assay window periods for application in organ donation and transplantation practice.

Current window period estimates are based both on an arbitrary sensitivity cut-off point and an assumption about the viral load of the inoculum.

In this paper we explored the impact of these assumptions on window period estimates for the most commonly used assay and platform in our jurisdiction.

We demonstrated that altering the sensitivity cut-off point to one based on the X95 LOD resulted in window period estimates that would account for some, but not all later phase HCV transmission events described in the literature. We subsequently modeled the impact of inoculum volume on the estimation of assay window periods and demonstrated that volume can have a significant impact on window period length.

As each organ has a potentially differing residual plasma volume, this study raises the concept of estimating window periods specific to both an individual virus and an individual organ. Whilst novel to organ donation, blood product specific window periods have been previously suggested for fresh frozen plasma.[29]

Intriguingly, within the organ donation literature, there may be an early signal of a correlation between organ plasma volumes and transmission risk. In a case series of 15 donors who transmitted HCV to at least one recipient, there was a trend towards lower transmission rates in lower volume organs (kidney 86% transmission, heart 76% transmission) when compared to higher plasma volume organs (liver 100% transmission, lung 100% transmission)[2].

However direct application of window periods based on in vivo plasma volumes is limited by a number of confounders, including dilution from operative flushing, perfusion practices, and presence of virus in extravascular compartments. The latter point being analogous to the pharmacological concept: a drug's volume of distribution, where some drugs are tightly restricted to the intravascular space, while for others, the majority of active particles are found in extravascular compartments. In the case of newly acquired HCV infection, the virus not only replicates in hepatocytes, but may also replicate in peripheral blood mono-nuclear lymphocytes, lymph nodes and various endocrine and exocrine organs.[30]

While in reality, window periods likely vary between organs transplanted there is currently insufficient knowledge to model these estimates with any degree of certainty.

We finally present a conservative set of assumptions based on a single inoculating virion capable of replication into the average adult plasma volume of 4,600 mL. Previous studies suggest this is the most likely inoculating dose for HIV and HCV infections acquired from non-medical injecting drug use using standard low dead space injecting equipment.[31] Adopting these values assumes that a transplantable organ is theoretically capable of transmitting infection immediately after infectious exposure of the donor, even though plasma viral load is unlikely to be sufficiently high enough to *average* 1 virion per organ-plasma-volume for several days unless there is a specific tissue tropism (see Supplementary Material).

Adoption of the "total body plasma" estimate, results in X50 window estimates that are universally longer than current TSANZ guidelines. Adoption of these estimates would result in an increase in the estimated residual risk of infection from increased viral risk donors, when calculated using the incidence rate-window period model, and an increase in the interval prior blood draw where increased viral risk behavior may result in undetected infection.

We demonstrate that adoption of X95 LOD is unnecessary in this instance, as modeling based on the total area under the curve suggests intervals slightly shorter than the X50 window period would still result in >95% of all potential window period infectious exposures being detected at screening.

Important assumptions underpin our modeling. We assume that in early infection, there is linear viral growth with fixed doubling times, conservatively that the minimum infective dose is a single viral particle, and we make inferences about the external validity of manufacturer reported assay performance, and our understanding of the viral concentration in international reference standards. These assumptions apply equally to previous estimates of window periods produced for assays by other manufacturers.

There has been significant progress in the management of donor derived blood borne virus infections in transplant recipients. Donor derived HCV infection can be cured[32] and donor derived HBV transmission can be prevented in recipients of non-liver transplants through antiviral treatment or by selection of recipients with adequate immunity.[33,34] Transmitted HBV can be managed with antivirals.[35–37] Unexpected donor derived HIV infection is extremely rare, and with treatment, recipients can expect to have a life-expectancy similar to patients without donor-derived HIV.[38–41]

Whilst the clinical consequence of donor derived infections has diminished with time, the need for timely identification of disease transmission, notification to biovigilance programs and timely initiation of therapy to prevent morbidity remains. Routine NAT screening of all transplant recipients, while routine in the US and now Europe,[42,43] has not been implemented in all regions of the world.[1] Ongoing reporting and publication of key intervals associated with unexpected transmission events will be key to defining "real-world" window periods for these assays.

Although the risk of unexpected disease transmission of blood borne viruses remains both small and manageable, our paper serves to highlight the inherent uncertainty involved in the estimation of NAT window periods.

Clinicians are cautioned against integrating NAT window period estimates from blood donor screening directly into their clinical practice. These estimates likely underestimate window periods in clinical

transplantation practice. Our paper reinforces that residual risk is a continuous phenomenon, and not simply limited to a specific interval defined by an assay's window period.

In summary, we have provided updated window period estimates for the most commonly used assay to screen Australian deceased organ donors. For some viruses, these estimates are now significantly shorter than national and international consensus intervals adopted by transplantation bodies. However, we also conclude that these well-established methods may only be valid for the blood transfusion sector and may have limited external validity for organ donation and transplantation. We have proposed a more conservative model.

Donation and Transplantation societies are now left in the unenviable position of determining the merits of retaining existing guidance, adopting conservative estimates such as ours, or serially extending window periods as new late phase transmission events are described. However continued application of assumptions designed to estimate the residual risk of infection from blood transfusions, no longer seems tenable.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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