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ORIGINAL ARTICLE

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International review of blood donation nucleic acid amplification testing

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Abstract

Background and Objectives: Nucleic acid amplification testing (NAT), in blood services context, is used for the detection of viral and parasite nucleic acids to reduce transfusion-transmitted infections. This project reviewed NAT for screening blood donations globally.

Materials and Methods: A survey on NAT usage, developed by the International Society of Blood Transfusion Working Party on Transfusion-transmitted Infectious

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Diseases (ISBT WP-TTID), was distributed through ISBT WP-TTID members. Data were analysed using descriptive statistics.

Results: Forty-three responses were received from 32 countries. Increased adoption of blood donation viral screening by NAT was observed over the past decade. NAT-positive donations were detected for all viruses tested in 2019 (proportion of donations positive by NAT were 0.0099% for human immunodeficiency virus [HIV], 0.0063% for hepatitis C virus [HCV], 0.0247% for hepatitis B virus [HBV], 0.0323% for hepatitis E virus [HEV], 0.0014% for West Nile virus [WNV] and 0.00005% for Zika virus [ZIKV]). Globally, over 3100 NAT-positive donations were identified as NAT yield or solely by NAT in 2019 and over 22,000 since the introduction of NAT, with HBV accounting for over half. NAT-positivity rate was higher in first-time donors for all viruses tested except WNV. During 2019, a small number of participants performed NAT for parasites (*Trypanosoma cruzi*, *Babesia* spp., *Plasmodium* spp.).

Conclusion: This survey captures current use of blood donation NAT globally. There has been increased NAT usage over the last decade. It is clear that NAT contributes to improving blood transfusion safety globally; however, there is a need to overcome economic barriers for regions/countries not performing NAT.

Keywords

blood, NAT, safety, transfusion, TTI, virus

Highlights

- Over the past decade, there has been increased adoption of nucleic acid amplification testing (NAT) to screen donations for transfusion-transmitted viruses.
- Globally, over 3100 NAT-positive donations were detected as NAT yield or solely by NAT in 2019 and over 22,000 since the introduction of NAT.
- NAT contributes to improving global blood safety.

INTRODUCTION

Nucleic acid amplification testing (NAT) detects targeted nucleic acid sequences in a sample with high sensitivity and specificity. NAT is used for screening blood donations for viruses and parasites globally, reducing the risk of transfusion-transmitted infectious diseases (TTIDs) and thereby providing an additional layer of blood safety [1]. NAT for blood donation was initially implemented for human immunodeficiency virus (HIV) and hepatitis C virus (HCV) in the 1990s, and soon after for hepatitis B virus (HBV) [2]. NAT is now also used in selected regions for other viruses including hepatitis E virus (HEV), West Nile virus (WNV) and/or Zika virus (ZIKV), as well as for parasites including *Babesia* spp. [3–9]. Given the detection of acute/incident infections, NAT is fundamental for tracking changes in the epidemiology and distribution of bloodborne infections over time.

Since the adoption of NAT for blood donation screening, there have been at least three international collaborative studies capturing global usage and yield of viral NAT in blood donations [2, 10, 11]. An increasing number of countries have participated, highlighting the increased adoption of NAT globally. The last survey was conducted by the International Society of Blood Transfusion (ISBT) Working Party on Transfusion-transmitted Infectious Diseases (WP-TTID) using data from donations collected during 2008 [2]. The findings of this previous survey provided evidence for increasing use of NAT to improve blood safety. Since the last survey, a number of changes impacting NAT have occurred, such as technological improvements in testing chemistries and automation. NAT has been expanded for use in molecular surveillance of infectious diseases and to screen for emerging pathogens transmitted by blood.

Over 10 years have passed since the last international NAT survey [2] and well over 20 years since NAT was first implemented [11]. Given this, the Virology and Surveillance, Risk Assessment and Policy subgroups of the ISBT WP-TTID developed and conducted a new survey, with the aim to capture the current use and safety benefits of NAT.

MATERIALS AND METHODS

This survey was based on questions used in the previous survey with appropriate modifications and additions (Data S1) [2]. Participants could complete the survey online through the Qualtrics flexible survey tool (qualtrics.com) or manually using a fillable PDF or Word document. The survey was executed in 2021–2023, but asked participants to provide data for 2019 (1 January–31 December). This year was selected because it was prior to the COVID-19 pandemic so as not to

capture any possible testing changes or impact on donor populations due to the pandemic. The survey focused on NAT of blood donations for clinical products but not plasma for fractionation.

The survey was first circulated through ISBT WP-TTID members on 13 May 2021, with two follow-up reminders (sent on 13 October 2021 and 2 February 2022). The major global suppliers of NAT assays for blood donation screening, Roche Diagnostics (Basel, Switzerland) and Grifols Diagnostic Solutions (Emeryville, CA, USA), were asked to encourage their customers to participate. The survey was publicized during the Global ISBT Virtual Congress in June 2022. Personal emails were sent in August 2022 to members of the WP-TTID who had not responded to the survey. Finally, the survey was again publicized during the 33rd Regional ISBT Congress in Gothenburg, Sweden, in June 2023. The data captured and presented here include all responses received up to 18 September 2023.

Duplicate responses were removed. Responses containing no answers to questions relating to NAT were also removed. A small number of responders provided incomplete answers to some questions or sections; in these instances, only responses that allowed interpretation (e.g., where both the number of donations tested by NAT and the number of NAT-positive donations were provided) were included in each analysis, hence differing numbers of responders throughout. Descriptive analyses were performed, with reported variables expressed as frequencies and percentages, and 95% confidence intervals (CI) calculated. Given that some regions within a country reported different responses to some questions, percentages were based on the proportion of survey responses, not the whole country. The incidence/prevalence of HIV, HCV and HBV for responder countries was obtained [12, 13]. Comparisons of incidence/prevalence between survey responders performing NAT and those not performing NAT were performed with a Mann–Whitney test, using GraphPad Prism.

This study was a review of operational processes and summary data without donation or donor identifiers, and therefore not considered research on human subjects. Therefore, ethical approval for human research was not required.

RESULTS

NAT usage, 2019

A total of 43 responses were received from 32 countries (Figure 1). The data from our survey represent results for 2019 from over 28 million donations and cover a population of over 1 billion people. There was a diverse geographical distribution of survey respondents, with the largest proportion from Europe ($n = 16$), followed by Asia and Western Pacific regions ($n = 14$), South America ($n = 5$), Africa ($n = 5$) and North America ($n = 3$).

Of the 43 survey responses, 38 indicated that they perform NAT for at least one virus (Table 1), representing 27 countries: Argentina, Australia, Belgium, Brazil, Canada, China, Colombia, Denmark, France, Germany, Greece, India, Ireland, Japan, New Zealand, Oman, Poland, Republic of Korea, Singapore, South Africa, Spain, Switzerland, Thailand, The Netherlands, United Kingdom, United States of America and Vietnam. HIV, HCV and HBV NAT was performed by the largest proportion of responders (88%, 84%, and 84%, respectively), followed by HEV and WNV (each 26%), and finally ZIKV (7%). Most participants used NAT that detected HIV-1 in combination with HIV-2; three responders performed NAT specifically for HIV-1, with one performing NAT for HIV-2 separately.

The five responders not performing NAT for HIV, HCV or HBV indicated economic reasons for the lack of testing. Responder countries not performing NAT had a higher incidence/prevalence of HCV and HBV compared to responder countries performing NAT (Table 2). One respondent not performing NAT indicated that implementation was planned for 2023.

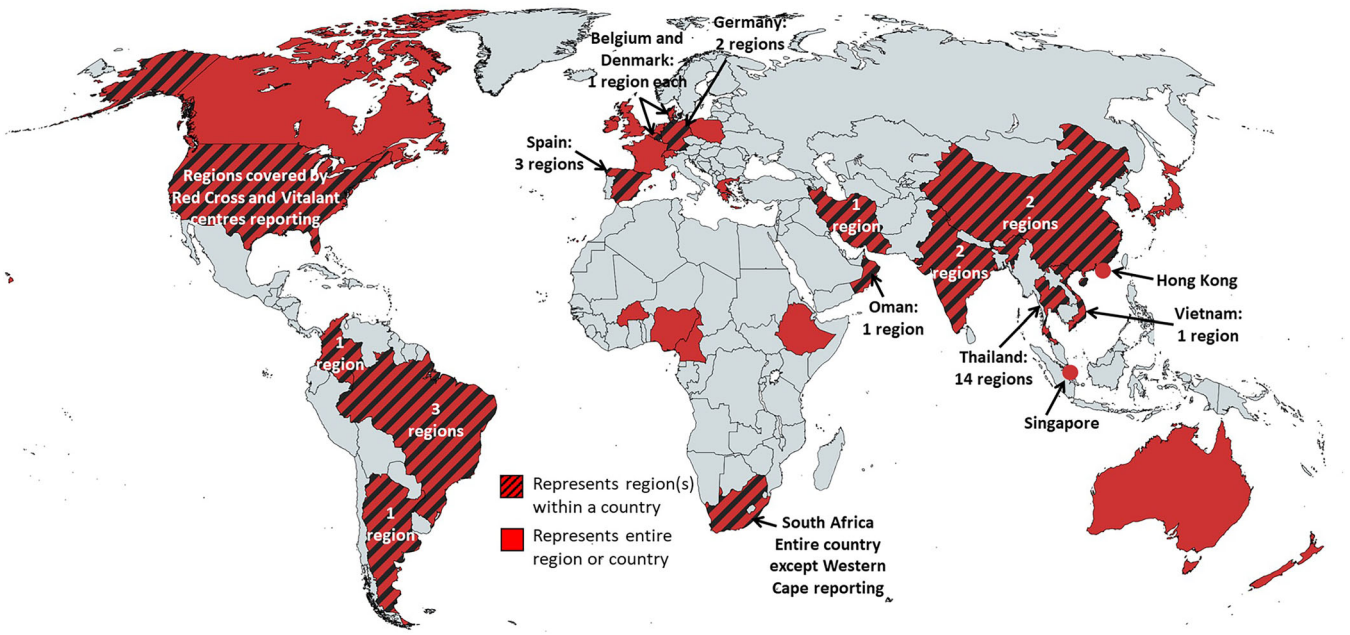

FIGURE 1 Geographical spread of survey respondents (prepared using mapchart.net).

TABLE 1 Survey responders performing blood donation NAT in 2019.

	HIV	HCV	HBV	HEV	WNV	ZIKV
Yes	38	36	36	11	11	3
No	5	5	5	30	30	38
No response	0	2	2	2	2	2
Proportion performing NAT	88%	84%	84%	26%	26%	7%

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HIV, human immunodeficiency virus; NAT, nucleic acid amplification testing; WNV, West Nile virus; ZIKV, Zika virus.

TABLE 2 Viral incidence/prevalence in the countries of survey responders performing NAT and those not performing NAT.

	HIV[a]	HCV[b]	HBV[c]
Incidence/prevalence (median)—NAT[d]	0.15% ($n = 23$)	0.30% ($n = 35$)	0.29% ($n = 34$)
Incidence/prevalence (median)—no NAT	0.19% ($n = 5$)	0.70% ($n = 5$)	1.32% ($n = 5$)
p-value	0.4375	0.0447	0.0073

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; NAT, nucleic acid amplification testing.
[a]HIV incidence, adults aged 15–49 per 1000 uninfected population, 2019 [12].
[b]Modelled viraemic prevalence, 2020 [13].
[c]Proportion of new cases of acute HBV in all sexes and ages per 100,000 people, 2019 [12].
[d]Data not available for all survey responder countries.

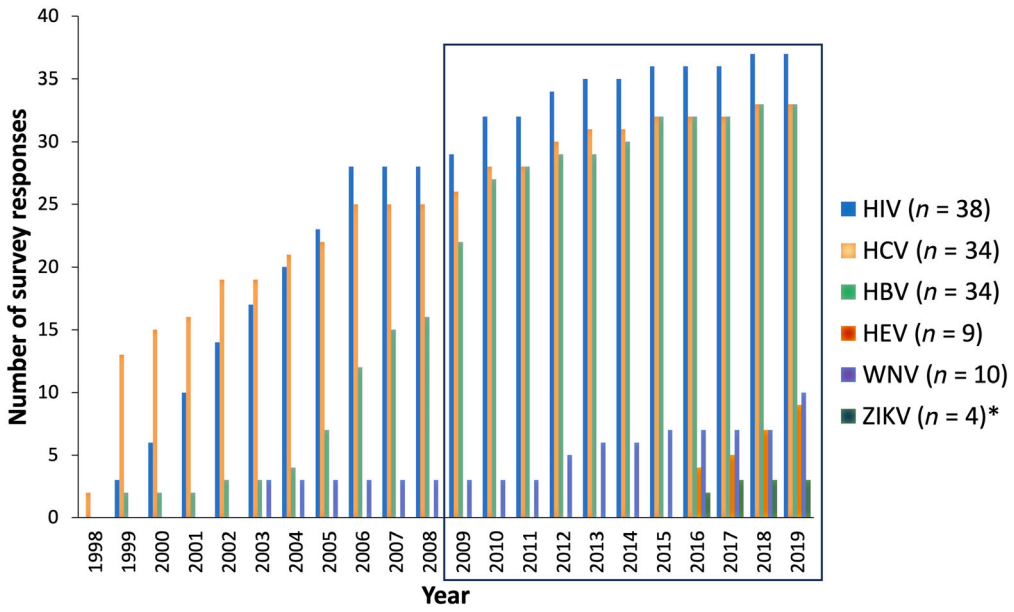

FIGURE 2 Implementation year of NAT for each virus. Box highlights time period since data were collected for the last ISBT NAT survey [2]. Data were not available from all respondents. *One survey responder indicated ZIKV NAT was used in 2016 only. HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HIV, human immunodeficiency virus; ISBT, International Society of Blood Transfusion; NAT, nucleic acid amplification testing; WNV, West Nile virus; ZIKV, Zika virus.

Date of NAT implementation

Data were collected for the previous NAT survey in 2008; since then, there has been an increase in the implementation of HIV, HCV and, later, HBV NAT among the survey responders (Figure 2). Since this time, only eight additional regions implemented NAT for these agents by 2019. Since 2008, there has been an increase in the adoption of NAT for other viruses; ZIKV, WNV and HEV testing have been implemented in four, seven and nine new regions or countries, respectively. The earliest adoption of ZIKV or HEV NAT was in 2016.

NAT-positive donations, 2019

In 2019, the proportion of donations positive by NAT (with or without detectable antibodies, if applicable) were 0.0099% (95% CI: 0.0095%–

TABLE 3 NAT-positive and NAT yield donations by region, 2019.

	Africa ($n = 1$)	Asia and Western Pacific ($n = 11$)	Europe ($n = 15$)	North America ($n = 3$)	South America ($n = 5$)	Total ($n = 35$)
Inhabitants supplied by blood operators (*n*)	54,000,000	>293,333,957[a]	250,543,947	366,156,716	>22,553,901[a]	>986,588,521[a]
HIV						
Donations tested (*n*)	949,121	11,118,151	8,764,993	6,668,100	349,295	27,919,660
NAT-positive donations (*n*)	2046	449	98	98	76	2767
NAT-positivity (rate[b])	2155.68	40.13	11.18	14.70	217.58	99.11
NAT yield[c] donations (*n*)	66	22	4	0	2	94
NAT yield[c] (rate[b])	69.54	1.97	0.46	-	5.73	3.37
HCV						
Donations tested (*n*)	949,121	11,183,633	8,764,973	6,668,100	349,295	27,915,122
NAT-positive donations (*n*)	90	847	269	498	48	1752
NAT positivity (rate[b])	94.82	75.74	30.69	74.68	137.42	62.76
NAT yield[c] donations (*n*)	3	38	1	4	0	46
NAT yield[c] (rate[b])	3.16	3.40	0.11	0.60	-	1.65
HBV						
Donations tested (*n*)	949,121	11,188,151	8,764,987	6,668,100	349,295	27,919,654
NAT-positive donations (*n*)	1088	4823	544	359	74	6888
NAT positivity (rate[b])	1146.32	431.08	62.07	53.84	211.86	246.71
NAT yield[c] donations (*n*)	227	1577	53	6	0	1863
NAT yield[c] (rate[b])	239.17	140.95	6.05	0.90	-	66.73
HEV						
Donations tested (*n*)	0	0	3,209,633	0	200	3,209,833
NAT-positive donations (*n*)	0	0	1037	0	1	1038
NAT positivity (rate[b])	-	-	323.09	-	5000.00	323.38
WNV						
Donations tested (*n*)	0	0	103,430	6,380,208	0	6,483,638
NAT-positive donations (*n*)	0	0	0	93	0	93
NAT positivity (rate[b])	-	-	-	14.58	-	14.34
ZIKV						
Donations tested (*n*)	0	129,983	0	5,779,697	0	5,909,680
NAT-positive donations (*n*)	0	2	0	1	0	3
NAT positivity (rate[b])	-	15.39	-	0.17	-	0.51

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HIV, human immunodeficiency virus; NAT, nucleic acid amplification testing; WNV, West Nile virus; ZIKV, Zika virus.

[a]Data were not available from all respondents.

[b]Rate is expressed per 1000,000 donations.

[c]NAT yield refers to samples that test positive by NAT only and not on other tests, if performed.

0.0103%; 2767/27,919,660) or 99 per million donations for HIV, 0.0063% (95% CI: 0.0060%–0.0066%; 1752/27,915,122) or 63 per million donations for HCV, 0.0247% (95% CI: 0.0241%–0.0253%; 6888/27,919,654) or 247 per million donations for HBV, 0.0323% (95% CI: 0.0304%–0.0343%; 1038/3,209,833) or 323 per million donations for HEV, 0.0014% (95% CI: 0.0011%–0.0017%; 93/6,483,638) or 14 per million donations for WNV and 0.00005% (95% CI: 0%–0.00011%; 3/5,909,680) or 1 per million donations for ZIKV (Table 3). The majority of donations tested by NAT were from repeat donors (Figure 3a). For HIV, HCV, HBV, HEV and ZIKV, there was a greater overall rate (per million donations) of NAT-positive donations from first-time donors, while the reverse was observed for WNV (Figure 3b); however, this pattern was not observed by all survey responders for HEV and WNV (data not shown).

NAT-yield donations, 2019

NAT yield refers to donations testing positive for NAT, but negative by serology, if performed, and can be reported for HIV, HCV and

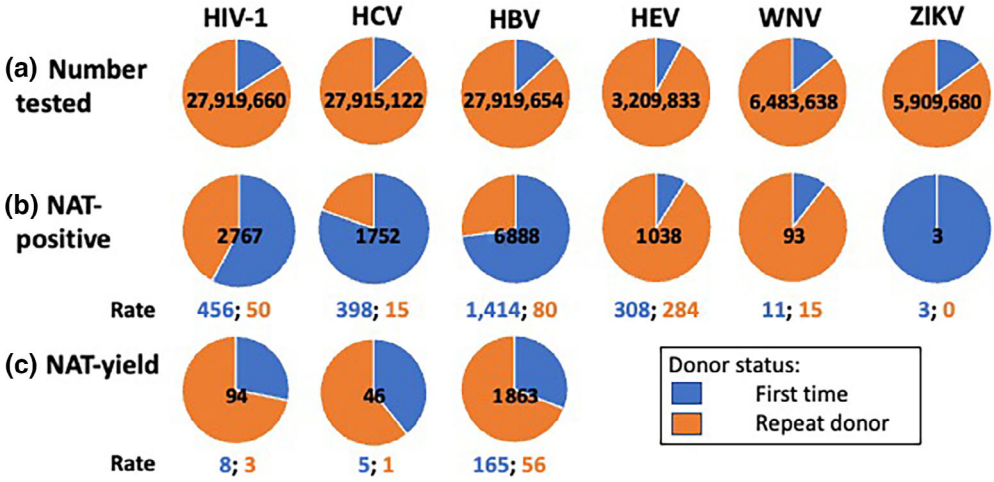

FIGURE 3 NAT-positive donations, 2019, by repeat and first-time donors, organized by (a) number of donations tested, (b) number of NAT-positive donations and (c) number of NAT-yield donations. Rates per million donations are provided. Data were not available from all respondents. HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HIV, human immunodeficiency virus; NAT, nucleic acid amplification testing; WNV, West Nile virus; ZIKV, Zika virus.

HBV. NAT-yield donations were identified for HIV ($n = 94$), HCV ($n = 46$) and HBV ($n = 1863$) (Table 3, Figure 3c). Although repeat donors made up the greatest proportion of NAT-yield donations, the NAT-yield rate per million donations was higher in first-time donors (Figure 3c).

Regional variation in NAT positivity and yield, 2019

Considerable variations in the NAT positivity and NAT-yield rates were observed between the different geographical regions (Table 3). The rate of HIV positivity was highest in donations from African donors, followed by those in South America, and the lowest in donations from Europe. HIV NAT-yield rates showed a similar trend; for 2019, none were detected in North America. For HCV, the NAT-positivity rate was highest in donations from South America, followed by those in Africa, and the lowest rate in donations from Europe. The HCV NAT-yield rate, however, showed a different trend, with no HCV NAT-yield donations from South America and the highest rate in the Asia and Western Pacific regions followed closely by Africa. The HBV NAT-positivity rate was highest in donations from African donors, followed by those from Asia and the Western Pacific, and the lowest rate in donations from North America. HBV NAT-yield rates showed a similar trend; however, none were detected in respondents from South America. The overall NAT positivity and NAT-yield rate were highest for HBV.

HEV NAT was performed on European and a very small number of South American donations, preventing yield to be compared by region. Nearly all WNV NAT was performed in North America; small numbers of donations were tested in Europe. All WNV NAT positives were observed in donations from North America. Again, nearly all ZIKV NAT was performed on donations from North American donors, with much smaller numbers performed in Asia and Western Pacific; however, the rates were higher in the Asia and Western Pacific regions compared to North America (albeit $n = 2$ vs. $n = 1$ positives per region, respectively).

NAT-positive donations and NAT yield since implementation

Similar to the 2008 survey, data were collected on NAT since its introduction until the end of 2019 in the regions and countries surveyed, to provide historical context on the value of NAT for blood screening (Table 4). Since implementation, over 517 million donations have been screened for HIV and HCV, with almost 370 million screened for HBV, reflecting its later implementation. HIV RNA was detected by NAT in 32,914 donations of which 1153 were NAT yields; HCV RNA was detected in 75,108 donations of which 1121 were NAT yields; and HBV DNA was detected in 68,096 donations of which 14,465 were NAT yields. The overall rate of NAT positivity and NAT yield was highest for HBV, which is similar to what was observed in 2008. The highest and lowest rates of NAT positivity and NAT yield since implementation were similar regionally to what was observed in 2008.

The number of donations tested for HEV, WNV and ZIKV were lower, reflecting their later date of implementation and regional and temporal use (Table 4). In Europe, where nearly all HEV NAT is performed, 1763 HEV NAT-positive donations were identified among nearly 8 million donations screened. Over 140 million donations, predominantly from North America, have been screened for WNV, with 3142 positive donations identified in North America and one in Europe. ZIKV NAT in Asia and Western Pacific, Europe and predominantly North America resulted in 589 positive donations from over 19 million donations.

TABLE 4 NAT-positive and NAT-yield donations by region, implementation to 2019.

	Africa ($n = 1$)	Asia and Western Pacific ($n = 11$)	Europe ($n = 15$)	North America ($n = 3$)	South America ($n = 5$)	Total ($n = 35$)
HIV						
Donations tested (*n*)	8,372,857	183,612,566	142,102,177	179,847,163	3,167,924	517,102,687
NAT-positive donations (*n*)	22,656	2575	1829	4794	1060	32,914
NAT positivity (rate[a])	2705.89	14.02	12.87	26.66	334.60	63.65
NAT yield[b] donations (*n*)	764	187	84	108	10	1153
NAT yield[b] (rate[b])	91.25	1.02	0.59	0.60	3.16	2.23
HCV						
Donations tested (*n*)	8,372,857	183,612,566	164,554,178	180,927,967	2,480,395	539,947,963
NAT-positive donations (*n*)	855	7926	10,968	54,153	1206	75,108
NAT positivity (rate[a])	102.12	43.17	66.65	299.31	486.21	139.10
NAT yield[b] donations (*n*)	50	262	191	614	4	1121
NAT yield[b] (rate[b])	5.97	1.43	1.16	3.39	1.61	2.08
HBV						
Donations tested (*n*)	8,372,857	152,221,471	102,928,968	103,272,077	2,418,536	369,213,909
NAT-positive donations (*n*)	12,462	33,093	15,975	5852	714	68,096
NAT positivity (rate[a])	1488.38	217.40	155.20	56.67	295.22	184.44
NAT yield[b] donations (*n*)	2318	11,116	897	124	10	14,465
NAT-yield[b] (rate[b])	276.85	73.03	8.71	1.20	4.13	39.18
HEV						
Donations tested (*n*)	0	0	7,721,643	0	337	7,721,980
NAT-positive donations (*n*)	0	0	1762	0	1	1763
NAT-positivity (rate[a])	-	-	228.19	-	2967.36	228.31
WNV						
Donations tested (*n*)	0	0	480,861	139,722,060	0	140,202,921
NAT-positive donations (*n*)	0	0	1	3142	0	3143
NAT positivity (rate[a])	-	-	2.08	22.49	-	22.42
ZIKV						
Donations tested (*n*)	0	383,148	19,800	18,898,123	0	19,301,071
NAT-positive donations (*n*)	0	10	147	432	0	589
NAT positivity (rate[a])	-	26.10	7424.24	22.86	-	30.52

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HIV, human immunodeficiency virus; NAT, nucleic acid amplification testing; WNV, West Nile virus; ZIKV, Zika virus.

[a]Rate is expressed per 1000,000 donations.

[b]NAT yield refers to samples that test positive by NAT only and not on other tests, if performed.

Residual risk estimates

To understand the different approaches used for calculating the residual transfusion-transmission risk for the different viruses tested by NAT, we asked whether such estimates were calculated in 2019 and, if so, which method was used. Approximately half of the participants indicated they did not perform such risk modelling calculations (Data S2). For those who did undertake these analyses, the classic incidence window-period model (based on repeat donor incidence and the pre-NAT infectious window period) [14] was used more often than the Weusten risk-day equivalent model [15] or the NAT yield and limiting antigen avidity (LAg) assay recent window-period ratio methods [16–18].

NAT for other agents

During 2019, NAT was performed for other agents (data not shown). Other TTIDs tested by NAT in 2019 included hepatitis A virus (HAV; $n = 6$), parvovirus B19 (B19; $n = 7$), cytomegalovirus (CMV; n = 1), human T-lymphotropic virus types 1 and 2 (HTLV-1/2; $n = 2$), *Trypanosoma cruzi* ($n = 1$), *Babesia* spp. ($n = 1$) and *Plasmodium* spp. ($n = 3$).

A number of responders indicated implementation or planned implementation of NAT for HAV, B19 and HTLV-1/2. Implementation of NAT for dengue, chikungunya and Zika viruses would be considered by a number of respondents if their regional epidemiological situation changes.

DISCUSSION

Our survey reports updated data on global blood donation NAT screening. We observed increased adoption of NAT for transfusion-transmitted viruses over the past decade, with an increase in HIV, HCV and HBV NAT usage and increased implementation of NAT for other viruses such as HEV, WNV and ZIKV. NAT-positive donations (including NAT yield and concordant NAT/antibody-positive donations) were identified for all viruses tested. Within the survey period of 2019, over 3100 NAT-positive donations were identified as NAT yield or solely by NAT. Since its introduction, over 519 million donations have been screened by NAT, with >22,000 donations identified as NAT yield or solely by NAT. HBV accounted for the majority of NAT-positive and NAT-yield donations, which is consistent with what has been reported previously in countries that do not perform anti-HBc testing [19]. Without NAT, these donations could have potentially resulted in TTIs in recipients of multiple components derived from each donation. NAT has thus been a significant contributor to improving blood transfusion safety globally. NAT, as an alternative to travel deferrals (e.g., for WNV in non-endemic settings), allows donations to be collected, rather than the deferral of donors, thus also contributing to sufficiency of supply. The main barrier for regions/countries not performing NAT was economic. For example, HIV, HCV and HBV NAT had previously been shown to be not cost effective in Zimbabwe [20]. Reducing cost and improving access to suitable assays for resource-limited countries may assist with adoption of blood donation NAT in such regions, further improving global blood transfusion safety, especially as incidence/prevalence of HCV and HBV was higher in responder countries/regions not performing NAT compared to those that do.

The overall HIV and HBV NAT-yield rates, per million donations, were higher in 2019 compared to 2008 (HIV: 3.37 vs. 1.93, respectively; HBV: 66.73 vs. 8.50, respectively), while HCV had a slightly lower NAT-yield rate (1.65 vs. 1.86, respectively). In Africa and the Asia and Western Pacific regions, the NAT-yield rates for all viruses was higher in 2019 than in previous periods, whereas for the other regions decreases were observed, for example, for HIV and HCV in Europe, as well as HCV and HBV in North America. Given that the global incidence of HIV, HBV and HCV decreased during this time [12, 13], increases in NAT-yield and NAT-positive rates (i.e., HBV) likely reflect improvements in NAT sensitivity. Significant regional variability in NAT-yield and positivity rates exists, reflecting differences in local viral epidemiology, highlighting the importance of tailoring blood safety initiatives to local situations.

During 2019, the majority of donations tested by NAT by survey responders were from repeat donors, reflecting the fact that this donor group makes up the majority of blood donors in survey responder regions. The overall NAT positivity rate for HIV, HCV and HBV was higher in first-time donors in 2019, similar to the previous study based on data from 2008 [2]. Although repeat donors make up the greatest number of HIV, HCV and HBV NAT-yield donations, which reflect their accounting for the greater proportion of donations tested, the NAT-yield rate per million donations was consistently higher in first-time donors for these three TTIDs. The large difference in NAT positivity between first-time and repeat donors suggests that repeat donors do self-risk assessments. Although the overall HEV NAT positivity rate in 2019 was higher in first-time donors, the reverse was observed by multiple responders; this appears to be driven by one survey responder that tested a large number of donations in 2019 and had a rate of HEV positivity higher in first-time donors. WNV was the only virus whose overall rate of NAT positivity in 2019 was higher in repeat donors (15 per million, compared to first-time donors, 11 per million), simply reflecting the fact that both first-time and repeat donors are at a comparable risk of being bitten by an infected mosquito; thus, since there are many more repeat donors, there are many more repeat WNV-positive donors. The number of ZIKV NAT-positive donations was small, with all three ZIKV NAT-positive donations in 2019 coming from first-time donors; however, repeat donors in North America were positive in previous years [8], and similar to WNV, donation status is not a contributor to positivity by a mosquito-borne agent.

Blood donation NAT was not restricted to these six viruses and was also performed on other agents in 2019, including HAV, B19, HTLV-1/2, *T. cruzi*, *Babesia* spp. and *Plasmodium* spp. A number of survey responders indicated planned implementation of NAT for other agents such as HAV, B19 and HTLV, or arboviral NAT, for some or multiple agents, if changes in their epidemiological situations occur. Moreover, laboratory-developed or research-use-only assays may be available in some jurisdictions, which were not captured in the present study. Such assays could be rapidly deployed in the initial response to emerging threats, negating the need to rely on commercial assays in such instances. With the emergence of different agents in different geographical regions, such in-house fit-for-purpose NAT assays may be the best first-line defence. In addition, there appears to be an increasing use of multiplex NAT assays, including those for emerging TTIDs. Given this ever-changing landscape, it is imperative that blood operators and TTID specialists continue to work together with commercial NAT assay manufacturers, such as through the activities of the ISBT WP-TTID, on a regular basis to ensure collaborative studies of performance of established and new NAT assays, such as in this report.

Our study has limitations. We report the results of NAT from 43 survey responders from 32 countries; other blood operators were invited to participate in this study, and many are performing blood donation viral NAT. For example, while we report no HIV NAT-yield donations in North America during 2019 among responders to our survey, such infections were detected during this time in donations given to organizations that did not contribute data to this survey and, in previous and subsequent years, for those in North America who did participate in this survey [21]. The results from our survey would be biased towards countries, regions or organizations actively involved in the ISBT WP-TTID and/or using Roche or Grifols NAT assays. We report the proportion of survey responders, rather than the country as a whole, given that some regions within a country reported different responses to some questions; but this does not affect NAT positivity or yield rates. Some countries or regions noted implementation or removal of NAT since 2019 (e.g., [22]), further highlighting the need to undertake surveys like this on a regular basis. We report "reactives"

and use that term as analogous to confirmed positives. Sensitivities of reported NAT assays and algorithms to reach a final consensus definition of positive were not defined by the survey or the ISBT, thus there will be differences for which we cannot control and may have an impact on our results. Thus, our results may have overestimated NAT yield, but even so, this should not have an impact on the trends that we reported here. Finally, we focused on blood donation viral NAT; given NAT is now also used for screening blood donations for parasites, such as *Babesia* spp. [9], future studies performed by the ISBT WP-TTID should be extended to cover all TTIDs.

To our knowledge, this is the largest survey of blood donation NAT to date and the only comprehensive snapshot of NAT usage in the past 10 years. Blood donation NAT usage has increased since its first introduction. Given the detection of over 22,000 NAT-only positive donations combined since its introduction, it is clear that NAT has played an important role in enhancing blood transfusion safety globally. Overcoming barriers in those countries/regions not performing NAT would undoubtedly offer the benefits of NAT, with potentially higher yield and impact on safety in low- and middle-income countries many of which have high burdens of TTIDs.

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H.M.F., C.O., B.C., M.B. and S.L.S. conceived the study and prepared the survey. H.M.F. prepared the first draft of the manuscript. All authors contributed to study design, data analysis, data interpretation and manuscript editing and approved the final version of the manuscript. Open access publishing facilitated by University of the Sunshine Coast, as part of the Wiley - University of the Sunshine Coast agreement via the Council of Australian University Librarians.

CONFLICT OF INTEREST STATEMENT

Helen M. Faddy has received research funding and/or honoraria from Grifols Diagnostic Solutions Inc. and Roche Diagnostic Solutions in the past. Brian Custer and/or the organization he is employed by has received research funding from Grifols Diagnostic Solutions Inc. and Roche Diagnostic Solutions. Susan Stramer has received research funding and/or honoraria from Grifols Diagnostic Solutions Inc. and Roche Diagnostic Solutions in the past. Christian Erikstrup has received unrestricted research grants from Abbott Diagnostics and Novo Nordisk, which are administered by Aarhus University Hospital and Aarhus University, respectively. Christian Erikstrup has not received any personal fees from these or other entities. Silvia Sauleda has received research funding from Grifols Diagnostic Solutions in the past. The remaining authors have no relevant conflict of interest (COI) to declare.

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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