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Blood donation screening for hepatitis B virus core antibodies: The importance of confirmatory testing and initial implication for rare blood donor groups

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Abstract

Background and Objectives: Exclusion of blood donors with hepatitis B virus (HBV) core antibodies (anti-HBc) prevents transfusion-transmitted HBV infection but can lead to significant donor loss. As isolated anti-HBc positivity does not always indicate true past HBV infection, we have investigated the effectiveness of confirmatory anti-HBc testing and the representation of rare blood groups in anti-HBc-positive donors.

Materials and Methods: Three hundred ninety-seven HBV surface antigen-negative and anti-HBc initially reactive blood donor samples were tested by five different anti-HBc assays.

Results: Eighty percentage of samples reactive in Architect anti-HBc assay were positive by the Murex assay and anti-HBc neutralization. Eleven out of 397 samples showed discordant results in supplementary testing from the Murex confirmatory test result, and five remained undetermined following extensive serological testing. Thirty-eight percentage of anti-HBc-positive donors identified as minority ethnic

groups compared with 11% representation in anti-HBc-negative donors ($p < 0.0001$); the frequency of the Ro blood group in anti-HBc-positive donors was 18 times higher in non-white ethnic groups.

Conclusion: Using two anti-HBc assays effectively enabled the identification of HBV-exposed and potentially infectious donors, their deferral and potential clinical follow-up. However, the exclusion of confirmed anti-HBc-positive donors will still impact the supply of rare blood such as Ro.

Keywords

anti-HBc testing, donor re-entry, ethnic groups, HBV screening, rare blood groups

Highlights

- A hepatitis B virus (HBV) core antibodies (anti-HBc) confirmatory algorithm using two different anti-HBc assays effectively delineates true and false reactivity; knowledge of false reactivity better guides deferral and follow-up strategies.
- Anti-HBc blocking and avidity assays were used to demonstrate the high efficacy of the anti-HBc confirmation strategy. These assays can be applied for confirmatory testing and to better understand the natural history of HBV infection.
- The high proportion of rare blood group donors positive for anti-HBc positive means that excluding them may impact the critical supply of rare blood. There may be a need to consider the implications of universal anti-HBc screening further.

INTRODUCTION

Around 2 billion people worldwide have serologic evidence of current or resolved hepatitis B virus (HBV) infection, indicated by the presence of antibodies to the hepatitis B core antigen (anti-HBc) [1] that typically persist lifelong following infection. It is established that HBV is transmissible by transfusion from blood donors who test negative for HBV surface antigen (HBsAg) but positive for anti-HBc [2, 3]. Many of these HBsAg-negative donors were found to have detectable HBV DNA and, therefore, recognized as having occult HBV infection (OBI) [4]. However, very low levels of HBV DNA are only intermittently detectable in donors' blood [5, 6]. Given the significant association of anti-HBc positivity with the presence of HBV DNA in the liver and the peripheral blood [7] and hence posing a risk of HBV transmission via blood transfusion, universal screening for anti-HBc has been implemented in many countries, including the United Kingdom [8]. However, transmission of HBV has been limited to anti-HBc-positive donors with low levels of antibodies against the HBV surface antigen (anti-HBs; <100 mIU/mL); hence, donors with higher anti-HBs levels can potentially be considered safe [9].

Identification of HBsAg-negative donors with potential long-term persistent HBV infections is further complicated by the limited specificity of anti-HBc assays used for screening, with up to 60% of initially reactive samples remaining unconfirmed [10–13]. The existence of an immunoglobulin M component in commercial anti-HBc total assays inherently restricts specificity further [14]. Specificity can be improved using robust confirmatory testing, including secondary anti-HBc assays or neutralization tests that measure the blocking of anti-HBc reactivity by using a soluble recombinant HBV core antigen (rHBcAg) [15]. Other means to identify if borderline anti-HBc positivity is a result of non-specificity or low levels of circulating anti-HBc [16] include the measurement of anti-HBc avidity, where the strength of binding of anti-HBc antibodies is measured by an avidity index (AI), which increases between acute and chronic HBV infections [17].

There is particular concern that the exclusion and deferral of anti-HBc-positive donors could negatively impact donor populations with rare blood groups associated with the absence of high-incidence red cell antigens. Indeed, it has been shown that most donors with OBI in the United Kingdom were born in areas of the world where HBV infection is endemic [18]. This is also where 55% of donors of Black ethnicities have the Ro blood subtype, a subtype only seen in 2% of all UK donors [19]. Rare blood phenotypes are in demand. The Ro subtype is frequently transfused to Ro patients with sickle cell disorder [19] to prevent stroke and crisis. Furthermore, the rare Rh type, rr, denotes a rare subtype of ABO group O Rh D negative, which is frequently transfused in emergencies [20]. The full impact of excluding anti-HBc-positive donors on the supply of units with rare blood groups in the United Kingdom remains unknown.

In this study, we have first investigated the effectiveness of two different anti-HBc assays to determine true anti-HBc positivity by applying an anti-HBc blocking assay and anti-HBc avidity test in a cohort of 397 blood donors reactive in one anti-HBc assay. We have also investigated the representation of rare blood groups in deferred anti-HBc-positive donors with high levels of anti-HBs antibodies.

MATERIALS AND METHODS

Clinical specimens

Three hundred ninety-seven ethylenediaminetetraacetic acid (EDTA) plasma samples were obtained from NHS Blood and Transplant (NHSBT) that had tested negative for HBsAg (Abbott Prism or Alinity S) and repeatedly reactive for anti-HBc (Architect, Abbott total anti-HBc II assay; signal to cut-off [S/CO] ratio ≥1.0 being considered reactive) between 16 June 2022 and 13 January 2023. These samples had also been screened negative for HBV DNA in pools of 24 (Roche Cobas 6800; 95% limit of detection [LOD] 1.4 IU/mL; calculated 95% LOD 33.6 IU/mL in individual donor level when tested in pools of 24) and for anti-HBs (Abbott Architect). Samples with anti-HBs levels <100 mIU/mL were tested further at NHSBT using a second anti-HBc assay (Murex total anti-HBc, DiaSorin) in duplicate, for antibodies against the HBV e antigen (anti-HBe; Biomerieux Vidas) and for HBV DNA using individual nucleic acid testing (NAT; Grifols Procleix Panther; 95% LOD 4.5 IU/mL). Anti-HBc positivity was defined as anti-HBc positive in the Architect and Murex assays and either anti-HBe or anti-HBs positive. In contrast, samples that were only anti-HBc positive in Architect and Murex but negative for other HBV markers were considered anti-HBc inconclusive. Architect positive but Murex negative were considered anti-HBc negative.

Clinical HBV testing at NHSBT was limited to samples with anti-HBs titres <100 mIU/mL as described above but not on samples with higher anti-HBs titres. To harmonize testing for the entire sample set regardless of anti-HBs titre, we tested samples with anti-HBs titres >100 mIU/mL for HBV DNA individually (Roche Cobas 6800; 95% LOD 1.4 IU/mL; $n = 250$). We then assayed all 397 samples by anti-HBc (Murex total anti-HBc, DiaSorin, Italy) and anti-HBe (FineTest Human HBeAb [hepatitis B virus E Antibody] ELISA kit, Wuhan, China) testing following manufacturers' instructions. Thirteen random anti-HBs <100 mIU/mL samples that were initially tested with the Murex assay at the clinical laboratory were also tested at the research laboratory, where a correlation of similar anti-HBc S/CO ratios with was found ($r = 0.940$, $p < 0.001$). Furthermore, testing of 19 random anti-HBs <100 mIU/mL samples (consisting of 10 anti-HBe negatives and 9 anti-HBe positives) with the FineTest kit revealed 100% concordant qualitative results with the VIDAS assay, and quantitative values strongly correlated ($r = 0.794$, $p < 0.001$). A further 26 HBV DNA-positive samples were used as additional positive controls in this study [21].

Demographic data were obtained from donors at the time of donation. The self-reported ethnicities of all 297,949 blood donors screened for anti-HBc in the 1 year since anti-HBc screening began in England from 30 June 2022 to 30 June 2023 were collated for comparison. The ethics statement is detailed in the supplementary material.

Assay development

In-house non-blocking and blocking assays were developed to study anti-HBc immune responses. For blocking anti-HBc reactivity using rHBcAg, 50 µL of each antigen dilution was pre-incubated with 50 µL of plasma dilution before plate washing and subsequent addition of immunoglobulin G (IgG). If specific anti-HBc IgG antibodies were present, these would be neutralized by rHBcAg. Assay development included standard curve calibration, negative run control and applying the blocking principle to the Murex blocking assay (Figures S1 and S2). Samples were diluted according to the screening Architect S/CO ratios aiming for OD values between 1.0 and 2.0 to normalize the denominator for calculating blocking percentages. An in-house avidity assay was also developed (Figures S3 and S4).

Rare donor analysis

Donors with rare blood types, alongside their ethnicities, were identified from the first anonymised 981 blood donors who were anti-HBc repeat reactive with anti-HBs ≥100 mIU/mL in the first 6 months of anti-HBc screening in England between 31 May 2022 and 30 November 2022. In the context of this study, rare blood types were defined as the following Rh genotypes: DcE/DcE (R2R2), Dce/dce (Ror, shortened to 'Ro') and dce/dce (rr). The number of positive anti-HBc and high titre anti-HBs donors of different ethnic groups within groups of rare Rh blood types was compared with the number of all active donors in the same ethnic groups from 30 June 2022 to 30 June 2023, excluding the 981 anti-HBc-positive donors.

Statistical analyses

Data normality was assessed with Shapiro–Wilks tests, where normal data are displayed as mean ± SD and non-normal data as median [interquartile range] where applicable. Spearman's correlation compared assay results between research and diagnostic laboratories. Fisher's exact tests compared categorical variables. Mann–Whitney U or t-tests compared continuous variables between two groups, whereas the Kruskal–Willis test or one-way analysis of variance (ANOVA) compared continuous variables with more than two groups. Receiver operating characteristic (ROC) analysis investigated the sensitivities and specificities of anti-HBc Architect ratios and anti-HBe titres that may predict anti-HBc positivity. All analyses were performed with GraphPad Prism (v10.0.2, LLC). Statistical significance was set at $p \leq 0.05$.

RESULTS

Validation of anti-HBc blocking assay

Validation of the assay was performed using 147 samples of known anti-HBc-positive status (all reactive in two anti-HBc assays [Abbot Architect and Murex] and one anti-HBe assay [Murex or Finetest]) and 55 anti-HBc-negative controls (weakly reactive in one anti-HBc assay [Abbott Architect] but negative in further anti-HBc assay

[Murex] and for anti-HBs as well as for anti-HBe), selected from 397 study samples. In addition, 26 previously characterized HBV DNA-positive samples were included as further positive controls [21]. Architect anti-HBc ratios correlated better with in-house IU/mL values than Murex anti-HBc ratios (Figure S5).

All anti-HBe-positive and HBV DNA-positive control samples were blocked by soluble rHBcAg in the HBcAg ELISA based on a greater than 50% reduction of anti-HBc binding (range 69%–94%; $n = 173$; Figure 1a). Architect, Murex and in-house anti-HBc titres were significantly higher ($p < 0.0001$) in the positive than in the negative controls (Figure 1b). However, two anti-HBc-negative controls were also blocked, with anti-HBc titres higher than the third quartile above the negative control mean (Figure 1). The remaining samples reactive in the Architect anti-HBc assay ($n = 195$) were then tested for blocking. Combining the results, significantly higher anti-HBc titres in Murex, Architect and rHBcAg ELISA assays were observed in blocked samples than unblocked or unreactive in the rHBcAg ELISA (Figure 1c; $n = 397$).

Serological correlates of confirmed anti-HBc positivity

A total of 317 of 397 anti-HBc initial Architect-reactive samples were positive in the Murex assay (80%). Of these, reactivity in the rHBcAg ELISA was blocked by soluble rHBcAg in 308 samples (308/317; 97%). However, reactivity was also blocked in two Architect-positive, Murex-negative, non-confirmed, presumed anti-HBc-negative samples (2/80, 2,5%; Figure 2). The neutralization observed in the latter two samples could not be blocked using a cell lysate from the strain DH5α of *Escherichia coli* with a comparable GCE33-YFP expression plasmid without rHBcAg, ruling out non-specificity from that source.

Anti-HBc avidity

Ninety-nine samples positive in the in-house anti-HBc assay were tested to investigate the relationship between anti-HBc avidity (AI) and anti-HBc reactivity. Reactivity in anti-HBc assays was highly predictive of avidity (Figure 3): anti-HBc ratios of ≥4.75 and ≥6 in the Architect and Murex assays, respectively, predicted AI values of >0.4 and excluded lower AI and anti-HBc titres ≥14 IU/mL in the in-house assay predicted AI values of >0.5 and excluded lower AI. When assessing the two discrepant Architect-positive but Murex-negative samples blocked in the in-house blocking assay, both had similarly low avidities irrespective of positivity in the Monalisa assay.

Overall, five (1%) anti-HBc initially Architect-reactive samples remained anti-HBc indeterminate after extensive confirmatory testing (Figure 2; Table 1). Seventy-nine percentage of anti-HBc initial Architect-reactive samples were genuine anti-HBc positive, whereas 20% were confirmed to be false positive. Ninety-eight (166/170) of isolated anti-HBc-positive samples concurrently Architect and Murex positive without anti-HBe antibodies were shown to be genuinely anti-HBc positive.

HBV DNA detection by individual NAT

Individual NAT identified one HBsAg-negative donor with anti-HBc antibodies positive for HBV DNA (Ct 36.4; IU/mL unavailable) with anti-HBs >100 mIU/mL from the 397 tested. This donor was known to have recently received an HBV vaccine booster.

Characterization of anti-HBc reactivities

Comparisons of initial anti-HBc screening ratios between anti-HBc confirmed positives, indeterminates and false positives revealed significant differences between groups in anti-HBc reactivity ($p < 0.0001$; Figure 4). There were significantly higher S/CO ratios for anti-HBc true positives (6.3 [4.0–7.4]) compared with anti-HBc indeterminates (1.5 [1.2–2.1]; $p < 0.0001$) and anti-HBc false positives (1.5 [1.1–1.9]; $p < 0.0001$) by post hoc Dunn test, whereas anti-HBc ratios between anti-HBc indeterminates and false positives were similar ($p > 0.999$). Comparisons of anti-HBe status in anti-HBc confirmed that positive donors showed significantly higher anti-HBc ratios in anti-HBe-positive than anti-HBe-negative donors (7.3 [6.3–7.6] vs. 4.6 [2.6–6.5], respectively; $p < 0.0001$).

Predictors of anti-HBc true positivity

The value of anti-HBc reactivity in the Architect screening testing and the presence of anti-HBe to predict true and false anti-HBc positivity were determined quantitatively by ROC analysis using anti-HBc Architect and anti-HBe sample-to-control ratios. An Architect ratio of 2.4 showed 87% sensitivity and 85% specificity in predicting anti-HBc true positivity (Figure 5), whereas an anti-HBe cut-off of 0.6 would have 86% sensitivity and 90% specificity.

Ethnic group comparisons

Sixty-one percentage of the 397 HBsAg-negative but anti-HBc repeat reactive donors were male, and 39% were female. Donors' median age was 47 years [36–59 years]. Fifty-four percentage described themselves as White British/Irish ethnicity, 13% as other White ethnicities, five donors did not disclose their ethnicities and the remaining 31% as other ethnic groups (specific ethnic groups found in Table S1). The proportion of minority ethnic groups in anti-HBc true-positive donors was significantly higher than their representation among all blood donors (38% vs. 11%; $p < 0.0001$; Figure 4). The proportions of 'Other Ethnic Groups' (38%) and 'Other White Background' (17%) donors in anti-HBc true-positive donors were also significantly higher than in anti-HBc indeterminate (11% minority ethnic groups and 0% Other White Background; $p < 0.0001$), respectively, and anti-HBc false-positive donors (7% minority ethnic groups and 3% Other White Background; $p < 0.0001$). Indeterminate and false-positive groups showed similar ethnic backgrounds ($p = 0.686$), and both groups showed similar proportions of minority groups to those of all blood donors ($p = 0.733$ and $p = 0.842$, respectively).

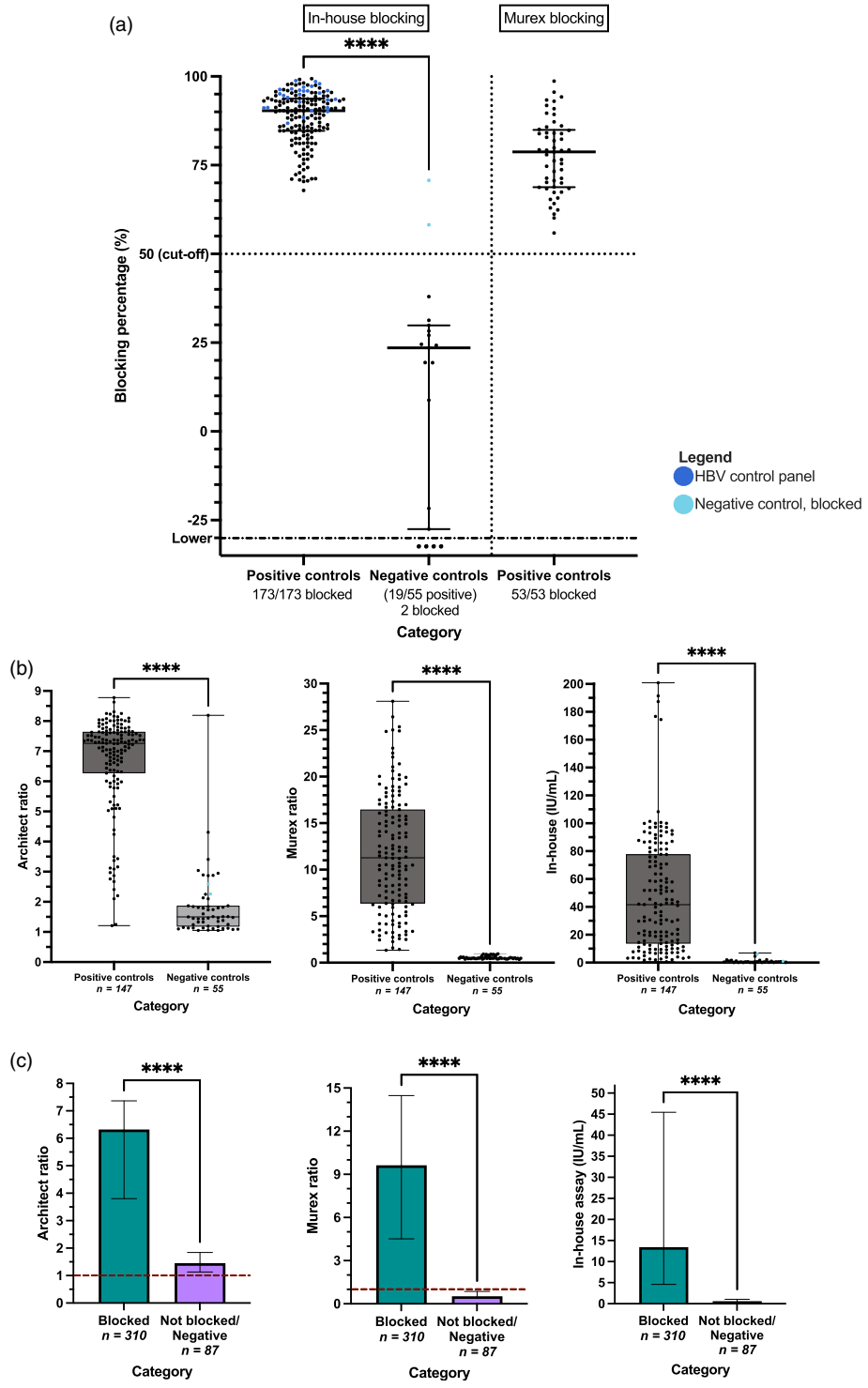


FIGURE 1 (a) Median [interquartile range] blocking percentages in all 173 hepatitis B virus (HBV) core antibody (anti-HBc)-positive controls (147 anti-HBc reactive in both screening assays, anti-HBe positive and 26 samples from the HBV control panel) and anti-HBc-negative controls (anti-HBc negative in the second screening assay, antibodies against the HBV e antigen (anti-HBe) negative and antibodies against the HBV surface antigen (anti-HBs) negative) tested in the in-house assay. Thirty-two of 51 negative controls were anti-HBc negative in the in-house assay, and the other 19 controls underwent blocking. The blocking percentages of 23 anti-HBc-positive controls tested in the Murex assay are also shown; (b) comparison of Architect, Murex and in-house anti-HBc ratios between positive and negative controls, showing all quartiles; (c) comparisons of the median [interquartile range] Architect, Murex and in-house anti-HBc ratios between donor samples that were anti-HBc blocked ($n = 310$) and samples that were either not blocked or were anti-HBc negative ($n = 87$). The dotted lines represent the 1.0 cut-off value for the anti-HBc screening assays. ****$p < 0.0001$ from Mann–Whitney U tests.

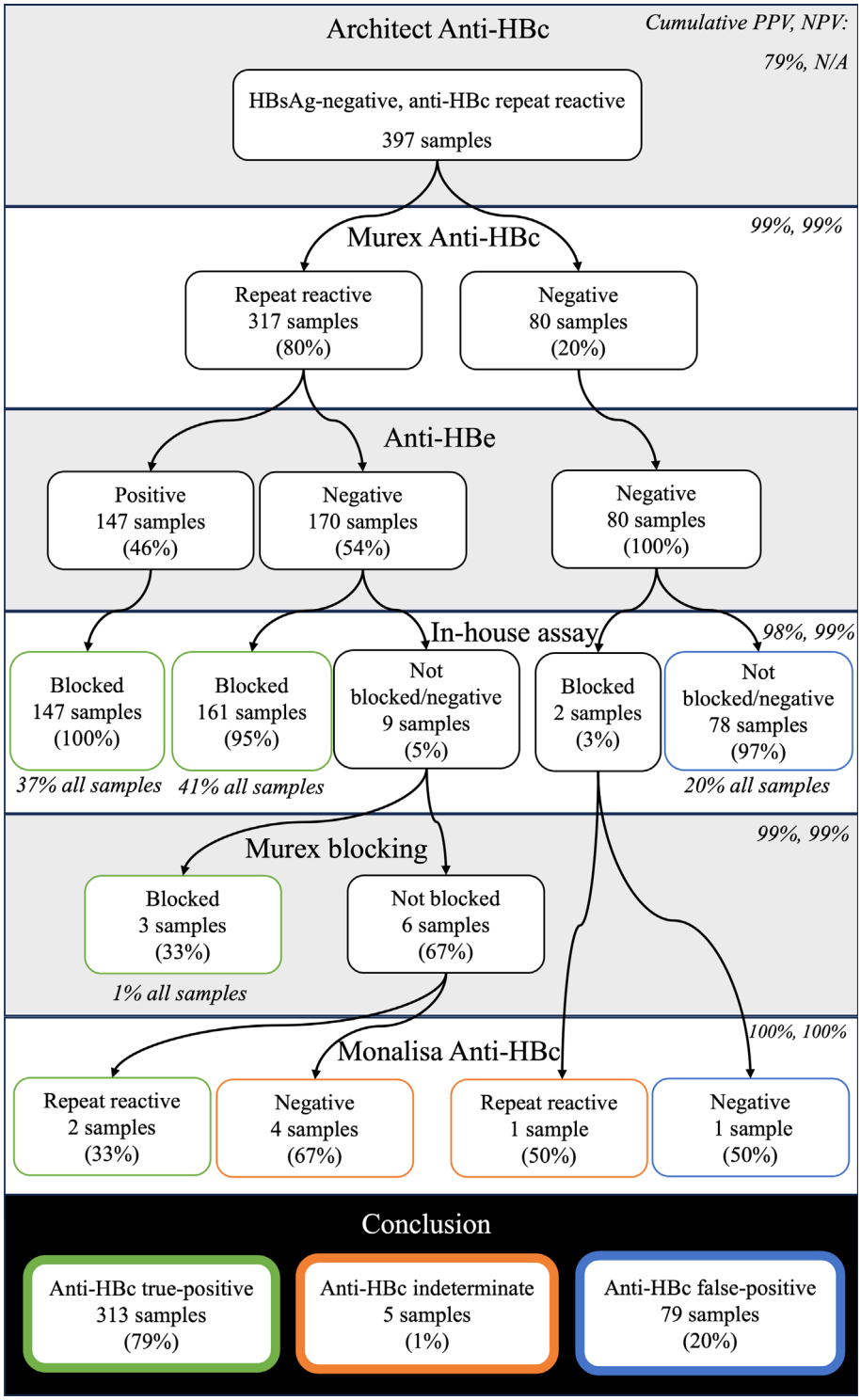


FIGURE 2 The flow diagram shows the delineation of hepatitis B virus (HBV) core antibody (anti-HBc) reactivity based on extensive testing of the 397 HBV surface antigen (HBsAg)-negative and anti-HBc initial reactive samples on the Architect assay. Testing included the Murex anti-HBc assay, anti-e antibody (anti-HBe) testing, in-house anti-HBc blocking assay, blocking on the Murex assay and the anti-HBc LIASION test. The cumulative positive predictive value (PPV) and negative predictive value (NPV) of the five anti-HBc assays to the true-positive and false-positive samples in the 'Conclusion' are shown in the top-right of each anti-HBc assay, where minimal changes are observed after the Murex assay. N/A: NPV not available since this was the first test performed in the algorithm.

Rare blood groups

Frequencies of selected blood groups were determined in the 981 anti-HBc-positive donors with anti-HBs ≥100 mIU/mL identified in England's first 6 months of anti-HBc screening. Of these, 301 (31%) had a rare blood type. There was a significantly higher proportion of rare blood group donors of White Other and 'Other Ethnic Groups' in anti-HBc-positive donors than in negative donors (29% vs. 7%,

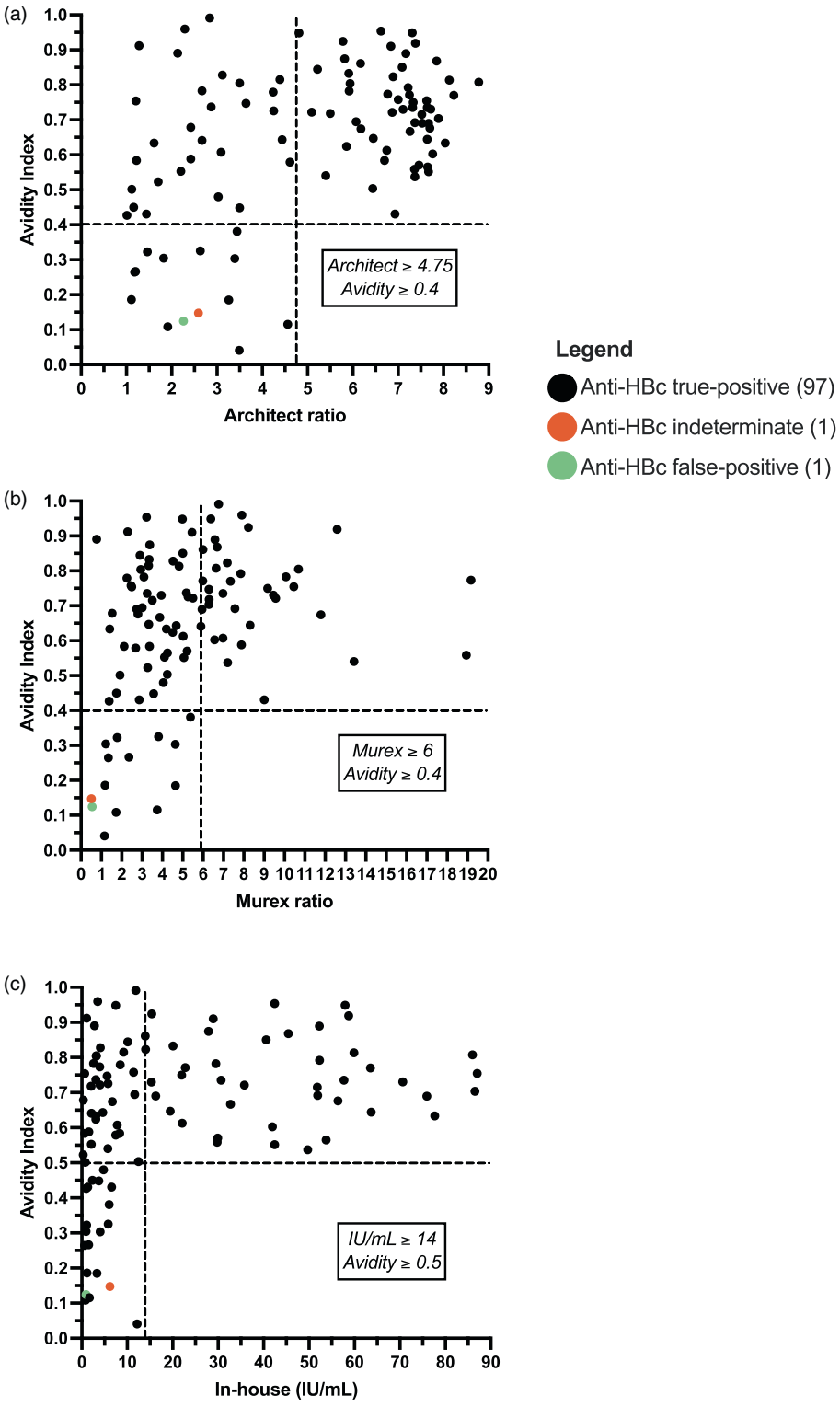


FIGURE 3 Correlation between avidity and hepatitis B virus core antibody (anti-HBc). (a) Architect signal to cut-off (S/CO) ratio ($r^2 = 0.196$), (b) anti-HBc Murex S/CO ratio ($r^2 = 0.109$) and (c) anti-HBc IU/mL from in-house assay ($r^2 = 0.006$), for all 99 samples tested for avidity and blocked. The dashed lines separate each anti-HBc ratio into a cut-off value for samples with lower avidity.

$p < 0.0001$; and 31% vs. 26%, $p = 0.033$; Table 2). Although there were similar proportions of R2R2 donors across ethnic groups ($p = 0.819$), there were significantly more Ro donors in anti-HBc-positive donors than anti-HBc-negative donors (8% vs. 3%, $p < 0.0001$), where the proportion of 'Other Ethnic Groups' Ro donors was 19 times that of White British/Irish donors. There was also a significantly higher proportion of rr donors of White Other ethnicities in anti-HBc-positive than all donors (25% vs. 2%, $p < 0.0001$).

TABLE 1 Serological and demographic characteristics of the 11 inconclusive samples identified from the in-house blocking assay.

Sample	Architect (ratio)	Murex (ratio)	In-house blocking (%)	Murex blocking (%)	Monalisa (ratio)	Anti-HBe (ratio)	Anti-HBs (ratio)[a]	Avidity index	Ethnicity
Pos-1	1.15	**5.94**	NA	**73**	**1.132**	0.55	>1000	NA	White British/Irish
Pos-2	1.20	**2.35**	24	**68**	**2.256**	0.55	>1000	0.266	Other Ethnic Groups
Pos-3	1.72	**3.23**	NA	**87**	**1.178**	0.62	0.00	NA	White British/Irish
Pos-4	1.70	**3.28**	−44	6	**1.938**	0.52	462	0.523	White British/Irish
Pos-5	2.42	**1.53**	21	38	**2.45**	0.39	2.18	0.678	White British/Irish
Ind-1	1.29	**2.24**	NA	16	0.709	0.00	0.29	NA	White British/Irish
Ind-2	1.32	**1.91**	NA	47	0.946	0.00	1.52	NA	White British/Irish
Ind-3	1.53	**1.23**	NA	11	0.686	0.46	0.00	NA	White British/Irish
Ind-4	3.61	**1.17**	NA	9	0.945	0.56	0.36	NA	White British/Irish
Neg-1	2.26	0.549	**58**	NA	0.532	0.41	1.06	0.124	White British/Irish
Ind-5	2.59	0.512	**71**	NA	**3.441**	0.41	2.30	0.148	White British/Irish
Median [IQR] values for all anti-HBc positives and negatives identified from in-house anti-HBc blocking assay									
Positives ($n = 308$)	6.34 [3.88–7.37]	9.68 [4.64–14.50]	87 [81–93]	79 [69–85]	-	0.95 [0.62–3.03]	471 [95–2000]	0.710 [0.566–0.805]	-
Negatives ($n = 78$)	1.45 [1.13–1.84]	0.502 [0.443–0.735]	9 [−23–24]	NA	-	0.43 [0.40–0.53]	1 [0–53]	NA	-

Note: Samples are labelled according to their final status: Murex blocking assay +/− Monalisa assay (Pos, Positive), not blocked on the Murex and negative on Monalisa (Ind, Indeterminate), Murex negative and positivity on Monalisa only (Ind, Indeterminate) and Murex negative and Monalisa positive (Neg, Negative). Bold values indicate positive values according to the assay protocols. Average ratios are shown in the table. NA indicates where the blocking and avidity assays were not done since anti-HBc was negative on the assay.

Abbreviations: anti-HBc, hepatitis B virus core antibodies; anti-HBe, hepatitis B virus e antibodies; anti-HBs, hepatitis B virus surface antibodies; IQR, interquartile range.

[a]Where anti-HBs titres were >1000 IU/L, 2000 IU/L was used as an approximation to obtain average values.

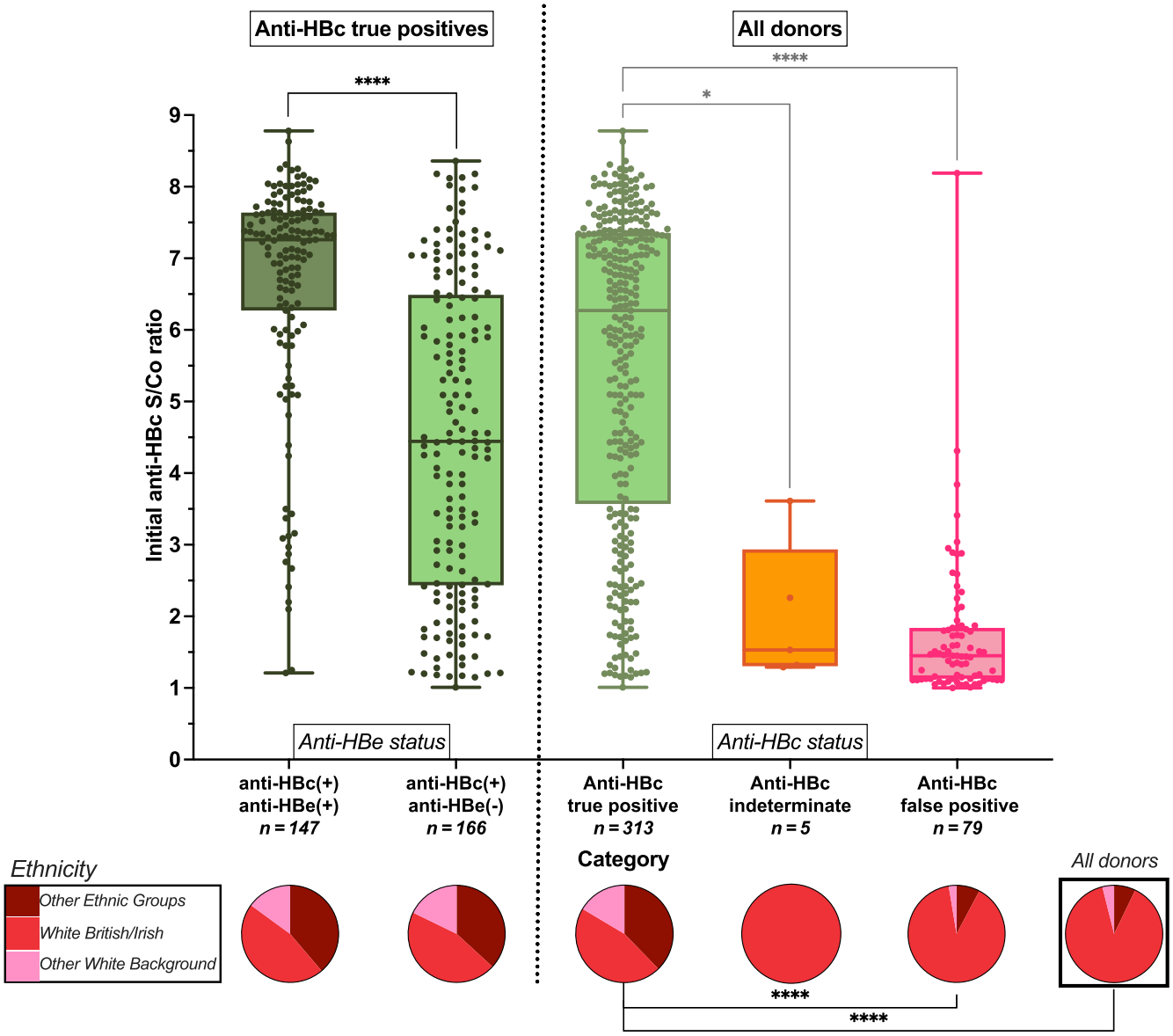
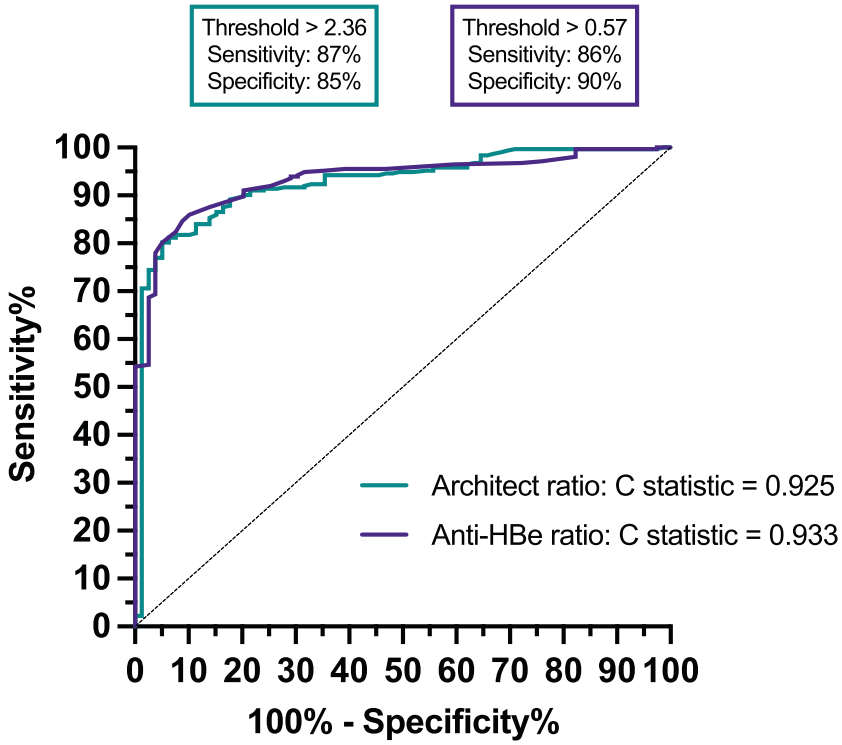


FIGURE 4 Comparisons of median [interquartile range] initial hepatitis B virus core antibody (anti-HBc) signal to cut-off (S/CO) ratios between the 397 donor samples categorized by anti-HBc genuine positivity, false positivity and indeterminate following the extensive testing detailed in Figure 2. The true-positive samples are further classified by HBV anti-e antibody (anti-HBe) status on the left hand of the graph, separated by a dotted line. The proportion of minority ethnic group donors for each category of donors is indicated, as well as from all donors in 2021. The Mann–Whitney *U* test compared anti-HBe status (darker coloured line), whereas the Kruskal–Willis test with post hoc Dunn's compared between the positivity categories (light coloured lines) and Chi-squared tests compared ethnic groups, where ***$p < 0.001$ and ****$p < 0.0001$.

DISCUSSION

This study investigated the specificity and effectiveness of current screening and confirmatory testing of donors for anti-HBc antibodies. The association between anti-HBc blocking and anti-HBc confirmation in the Murex assay demonstrated the latter's efficacy in delineating true and false anti-HBc-positive donors. Compared with the 80% specificity of the Architect assay, confirmation in the Murex assay reduced false positivity to around 1% of initially reactive donors (4/317) and 1% false negativity (1/80). Confirmation of anti-HBc reactivity showed a positive predictive value of 99% on Architect anti-HBc-positive samples. Determination of the true seroprevalence of anti-HBc positivity in donors enabled the investigation of the effects of anti-HBc-positive donor exclusion on the blood supply.

Specificity of anti-HBc testing is important and will help in communicating to donors if they have had a past HBV infection and providing appropriate advice about their future risk of developing HBV-associated progressive liver disease. HBV infection still carries a stigma, and many individuals deny having had a previous HBV infection, especially when the source of infection might simply relate to



Sensitivity (%)	Specificity (%)	Threshold anti-HBc S/Co ratio
95	53	1.48
90	80	2.10
87	85	2.36
82	90	2.88
77	95	3.40
2	100	8.21

Sensitivity (%)	Specificity (%)	Threshold anti-HBe S/Co ratio
95	68	0.47
90	80	0.55
86	90	0.57
80	95	0.60
54	100	0.83

FIGURE 5 Receiver operating characteristic analysis to investigate Architect signal to cut-off (S/CO) ratio (green) and HBV anti-e antibody (anti-HBe) ratio (purple) thresholds that may predict HBV anti-core antibody (anti-HBc) true positivity. For this analysis, anti-HBc true positives and true negatives (false positives) were utilized from this study's 397 samples, excluding the five indeterminate samples previously shown in Figure 2. The 90%/95% sensitivity and specificity threshold values for anti-HBc and anti-HBe are shown in the tables below the plot and 100% specificity thresholds for anti-HBc positivity.

them being born in HBV high-endemicity areas. Our application of blocking and avidity assays showed that positivity in two anti-HBc assays strongly predicted true anti-HBc-positive status, irrespective of assay S/CO ratios or anti-HBe/anti-HBs status. These results are highly relevant in interpreting isolated anti-HBc reactivity in donors or patients in general. Supplementary assays such as anti-HBc blocking may differentiate between past HBV infection and false reactivity in HBV-naïve donors [22] and provide an IgG-specific measurement that may increase specificity compared with assays measuring total anti-HBc. Testing algorithms using two different anti-HBc assays would not only reassure blood donors but would also help clinicians who need to balance their decisions on starting antiviral prophylaxis based on isolated, non-confirmed anti-HBc results only, as recommended by the European Association for the Study of the Liver (EASL) [23]. This testing algorithm may also be validated in other settings with alternative anti-HBc assays to provide equivalent confirmation of anti-HBc reactivity. Another strategy to identify false reactive donors would be utilizing a threshold value [13]. We found that below a cut-off value of 2.36, the Architect anti-HBc II assay showed high sensitivity and specificity in predicting anti-HBc true reactivity. Using a threshold value is supported by our finding that samples with higher anti-HBc titres showed consistently higher high functional affinity (avidity); the demonstration of maturity of anti-HBc antibodies is consistent with genuine past HBV infection.

The rate of anti-HBc false reactivity of 20% on the Architect anti-HBc II assay, found in this study following extensive testing, is much

TABLE 2 Comparisons of the proportion of ethnic groups between anti-HBc-positive donors ($n = 981$) and anti-HBc-negative donors ($n = 794{,}861$) for all rare blood types and each Rh blood type: R2R2, Ro and rr.

	Ethnic group			
	White British/Irish, *n* (%)	White other, *n* (%)	Other ethnic groups, *n* (%)	All groups, *n* (%)
All rare blood types				
Anti-HBc pos	119 (31%)	67 (29%)	115 (31%)	301 (31%)
Anti-HBc neg	200,019 (29%)	2666 (7%)	18,436 (26%)	221,121 (28%)
p-value	0.431	<0.0001	0.033	0.050
R2R2				
Anti-HBc pos	8 (2%)	6 (3%)	6 (2%)	20 (2%)
Anti-HBc neg	13,805 (2%)	779 (3%)	1159 (2%)	15,743 (2%)
p-value	0.855	0.46	>0.999	0.819
Ro				
Anti-HBc pos	3 (1%)	3 (1%)	82 (19%)	88 (8%)
Anti-HBc neg	15,372 (2%)	971 (2%)	10,494 (15%)	26,837 (3%)
p-value	0.055	0.385	0.014	<0.0001
rr				
Anti-HBc pos	108 (28%)	58 (25%)	27 (7%)	193 (20%)
Anti-HBc neg	170,842 (25%)	916 (2%)	6783 (10%)	178,541 (22%)
p-value	0.14	<0.0001	0.156	0.039

Note: The number of donors in each ethnic group for each blood type is shown, as well as the proportion of those donors representing all donors in that ethnic group for anti-HBc positive or negative donors. Fisher's exact tests were used to compute *p*-values.
Abbreviation: anti-HBc, hepatitis B virus core antibodies.

higher than the 6% rate in a previous study in a smaller population of 79 initially reactive samples [24]. Our rate is comparable to a German study of 22% using a confirmatory strategy of two older anti-HBc platforms [12], suggesting minimal changes in the false positivity rate of anti-HBc screening assays over time despite claimed improvements.

Our findings also show that all anti-HBe positivity was completely concordant with confirmed anti-HBc positivity and that anti-HBe positivity can, therefore, be utilized as a specific marker for past HBV infection [11] but not as a marker for OBI since most OBI cases are anti-HBe negative [21]. However, we suggest that the 0.6 to 1.0 range of anti-HBe results may constitute a zone of uncertainty: an anti-HBe S/CO threshold of 0.57 had high sensitivity and specificity in predicting anti-HBc true reactivity, and all donations with high 'negative' anti-HBe ratios above 0.83 were genuine anti-HBc positives. Furthermore, negativity in the anti-HBe assay has less predictive value; based on anti-HBc confirmatory testing, we found that the sensitivity of anti-HBe as a biomarker to identify resolved infection was limited. This may result from an increased prevalence of HBeAg-deficient HBV variants [13], the natural disappearance of detectable anti-HBe antibodies following recovery from infection and the possibility that anti-HBe antibodies may never develop during the natural history of HBV infection [25]. There is a need for alternative biomarkers; we have plans to investigate the potential use of new biomarkers, such as HBV core-related antigen (HBcrAg) [23, 26], for serological testing.

A further question is whether deferring anti-HBc-positive donors significantly impacts the supply of units with rare blood groups. We found that 38% of anti-HBc-confirmed-positive donors (in the absence or presence of other HBV markers) comprised minority ethnic groups, consistent with previous information from donors originating from countries with endemic patterns of HBV infection [27]. These donors are more likely to be Rh type D positive [28]; we additionally found that rare Rh phenotypes were more common in anti-HBc-positive donors from minority ethnic groups, whereas the proportion of rare groups in White British/Irish anti-HBc-positive donors was comparable to HBV-unexposed donors. The extensive minority ethnic group background of deferred anti-HBc-positive donors with high anti-HBs levels (≥100 mIU/mL) is more likely to have the rare Rh phenotypes Ro and rr that are critically needed for patients with sickle cell disorder to lower the risk of alloimmunisation.

The potential acceptance of anti-HBc-positive blood donors with high anti-HBs titres has been previously considered a safe means to maintain an adequate blood supply with sufficient rare blood types. However, we found one anti-HBc-positive donor with high anti-HBs levels (vaccine-boosted) of 'Other Ethnic Groups' to have detectable viraemia out of 397 donors screened for HBV DNA, although whether infectivity in this donation would have been neutralized by high levels of anti-HBs remains unknown. It is important to note that HBV transmission from anti-HBc-positive donors with high anti-HBs levels is rare and hence assumed to be a very low risk [9].

In conclusion, the development and application of anti-HBc blocking and avidity assays combined with testing for anti-HBe demonstrated relatively high specificity and sensitivity of the Murex anti-HBc assay for confirming donors reactive from screening with the

Abbott Architect assay. Indeed, a relatively simple confirmatory algorithm with two anti-HBc assays would almost eliminate false-positive results. The impact of anti-HBc screening and excluding all positives to rare blood group types needs further consideration.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The original datasets used and analysed in 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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