Excluding Occult Hepatitis B Infection before Assigning False-Positive Status to Non-Repeatable NAT Reactivity: Concerning Stolz et al. “Safe-Testing Algorithm for Individual-Donation Nucleic Acid Testing: 10 Years of Experience in a Low-Prevalence Country” [Transfus Med Hemother. 2019 Apr;46(2):104–10]

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Dear Editor,

Stolz et al. in their recent article [1] outlined the algorithm used by the Interregional Blood Transfusion Swiss Red Cross (Bern) for investigating samples that are initially reactive on individual-donation nucleic acid testing (ID-NAT), using the Procleix Ultrio or cobas MPX assays. If samples were negative on two further ID-NAT replicates (non-repeat-reactive, NRR) and also non-reactive on routine serological donor screening (which does not include anti-HBc), they were deemed to be NAT false positive and the donation was released for use with no further testing. Using a 10-year study period representing 1.8 million screened donations, the authors followed up 1,267 donors who tested NAT NRR and subsequently made at least one donation. All but one (who acquired acute hepatitis B virus [HBV] infection several years later) remained serology and ID-NAT negative. On the basis of these results, the authors conclude that their algorithm interdicted all true HIV-, HBV-, and HCV-positive donations and therefore can safely salvage false positive donations in countries with a low prevalence and incidence of these viruses.

This analysis by Stolz et al. [1] demonstrates that NAT NRR results in their donor population are unlikely to represent early window period infections, at least among

those donors available for follow-up. However, we believe some caution is required as the authors' algorithm and follow-up analysis is not sufficient to exclude occult HBV infection (OBI) among NRR donors.

It has been well documented that a subset of donors with non-repeatable and/or non-discriminated reactivity (NDR) on ID-NAT, including in low-prevalence countries, represent possible OBI [2–4]. As reviewed by Kiely et al. [2], there are at least two lines of evidence to support this. Firstly, the detection rate of anti-HBc is significantly higher in NRR/NDR donors compared to NAT non-reactive donors, for example, 12.2% in Ultrio NRR donors versus 6.0% in non-reactive donors (South Africa) [4], 13% Ultrio NDR and 57% Ultrio Plus NDR versus 6.8% in random donors (New Zealand) [3], and 7.6% Ultrio Plus NDR versus approximately 2.2% in non-reactive donors (Australia) [2]. Secondly, a proportion of these anti-HBc-positive donors are confirmed as OBI when retested using an alternative high-sensitivity NAT assay, or with additional replicates on the same assay [3, 5]. Therefore, NDR donations in Australia are tested for anti-HBc, and if positive, also undergo alternative NAT testing at an external reference laboratory. In addition, the NDR donation is discarded due to the risk

of HBV presence and transfusion-transmission. During the most recent 4 years of experience in Australia (2015–2018, 5.3 million screened donations), 941 donations were initially reactive on Ultrio Plus ID-NAT but non-reactive when retested on the same assay and on the HBV discriminatory assay. All were HBsAg negative. Additional testing documented that 20.83% ($n = 196$) had detectable anti-HBc. Of these, 12 (6.12%) were confirmed as OBI following detection of HBV DNA using either the Roche cobas AmpliScreen (2/82, 2.4%; HBV 95% limit of detection [LOD] 4.4 IU/mL) or cobas MPX (10/114, 8.8%; 95% LOD 1.4 IU/mL).

Individuals with OBI usually have very low viral loads, which is commonly associated with discordant HBV DNA results because of the sampling/Poisson effect [3, 5, 6]. For this reason, many blood services employ a range of alternative approaches to confirm or exclude OBI following initially reactive NAT results, including testing multiple replicates on the screening assay, use of alternative high-sensitivity NAT assays, testing of the associated plasma component and archived samples from the donor, and concentration of HBV viral particles from larger volumes of plasma [7]. Additionally, a recent study has reported acute transfusion-transmitted HBV in multiple recipients (5 confirmed, 3 probable, and 1 possible) who received blood components from donations that were ID-NAT negative on the Ultrio or Ultrio Elite ID-NAT assays [8]. The 3 implicated donors, all of whom were anti-HBc positive, were never reactive on the NAT screening assay (previously or at follow-up), and viral particle concentration was required to detect HBV DNA and confirm OBI during the recipient-triggered lookback investigations. The authors calculate the infectious dose for OBI as 16 copies, which would require NAT assays to have an LOD of ≤ 0.8 copies/mL [8] (lower than currently available NAT assays), although sampling effect may still cause OBI donations to elude screening. Given that transfusion-transmission of HBV (TT-HBV) is possible from ID-NAT-negative donations, and our confirmatory testing has documented that HBV DNA is confirmed as present in a proportion of our anti-HBc-positive ID-NAT NRR/NDR donations, then it is likely that this subset represents a significant TT-HBV risk.

We believe that OBI in donors who test NAT NRR can only be excluded with a reasonable level of confidence if anti-HBc testing is performed and negative. Similarly, in the absence of anti-HBc testing, a large number of subsequent donations without NAT reactivity for each followed-up donor would be necessary to suggest with confidence that OBI is unlikely, since the probability of detecting a low viral load increases with the number of tests performed. OBI donors with more than 10 previous ID-NAT-negative donations (Ultrio or Ultrio Plus) are not uncommon in Australia. Stolz et al. [9] have previously

reported an incremental transmission risk of only 1 in 4.1 million ($<10\%$ of total HBV residual risk) from releasing NRR donations, but this modelling did not account for OBI risk. Additionally, as these donations were not tested for anti-HBc and therefore the prevalence is unknown among this group, it is unclear how an accurate assessment of OBI risk from NRR donations could be made. The algorithm presented by Stolz et al. [1] would not be appropriate in Australia because of the risk of TT-HBV, despite a similarly low anti-HBc prevalence of approximately 2% among Australian and Swiss blood donors [2, 10]. We believe that anti-HBc testing for NAT NRR donations is an efficient and cost-effective way to help reduce the associated risk of OBI, and anti-HBc non-reactivity should be a minimum requirement prior to release of fresh blood components associated with NAT NRR results.

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The authors have no conflicts of interest to declare.

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Author Contributions

All authors approved the final version and agree to be accountable for all aspects of the work. Additional contributions are as follows: C.E.S. initially identified the risk of OBI potentially missed by the Stolz et al. algorithm [1], acquired and analyzed the new data here presented, drafted the work, and consolidated critical feedback. A.C. contributed to the acquisition and analysis of the new data presented and critically reviewed the manuscript. V.C.H., P.K., C.R.S., and M.W. made substantial contributions to the conception of the work and critically revised the manuscript.

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