Hepatitis B virus nucleic acid amplification testing of Australian blood donors highlights the complexity of confirming occult hepatitis B virus infection

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BACKGROUND: We present an analysis of the first 2 years of hepatitis B virus (HBV) nucleic acid testing (NAT) of the Australian donor population.

STUDY DESIGN AND METHODS: Between July 5, 2010, and July 4, 2012, all blood donations were screened for HBV DNA and hepatitis B surface antigen (HBsAg). Donors who tested HBsAg negative but HBV NAT positive were assessed as occult hepatitis B infections (OBI) if reactive for antibodies to HBV core antigen (anti-HBc). Donors who were anti-HBc reactive but with nonrepeatable or nondiscriminated NAT results were assessed as HBV inconclusive pending follow-up testing.

RESULTS: During the study period a total of 2,673,521 donations were screened for HBV. Forty-two chronic OBI infections (5.55/100,000 donors) were identified compared to eight acute serologic window period infections (1.06/100,000 donors). Of the 42 OBI cases, 23 (54.8%) were detected the first time they were screened for HBV DNA while 19 (45.2%) gave one or more HBV NAT–nonreactive results before detection. Of 68 donors initially assessed as HBV inconclusive and available for follow-up, 10 later confirmed as OBI cases while 51 were NAT nonreactive but remained anti-HBc reactive and OBI could not be excluded.

CONCLUSION: This study demonstrated a substantially higher prevalence of OBI compared to acute serologic window period HBV infections in Australian blood donors. Follow-up testing of OBI cases indicates that HBV DNA is often only intermittently detectable in OBI, highlighting the importance of including anti-HBc to optimize the HBV testing algorithm.

In June 2000 the Australian Red Cross Blood Service (Blood Service) implemented nucleic acid testing (NAT) for human immunodeficiency virus type-1 (HIV-1) and hepatitis C virus (HCV). More recently, after technologic advances in NAT assays and automated testing platforms with improved operating efficiency and process control, the Blood Service implemented a “triplex” NAT assay that included the simultaneous detection of hepatitis B virus (HBV) DNA in addition to HIV-1 and HCV RNA. Before the implementation of HBV DNA screening, detection of HBV infection in Australian blood donors was based primarily on screening for hepatitis B surface antigen (HBsAg), considered to be a serologic marker of active HBV replication. In addition, because HBsAg may not always be detected in chronic HBV infection, donors who disclosed a history of hepatitis or jaundice were also tested for HBV DNA, antibodies to hepatitis B core antigen (anti-HBc), and antibodies to hepatitis B surface antigen (anti-HBs), regardless of their HBsAg.

ABBREVIATIONS: ChLIA = chemiluminescent immunoassay; ID = individual donor; OBI = occult hepatitis B virus infection.

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result. Donors with a history of hepatitis or jaundice who tested anti-HBc reactive were eligible to continue donating if they did not have detectable HBV DNA or HBsAg and their anti-HBs quantitation was greater than or equal to 100 IU/L.

As with HIV-1 and HCV NAT, one of the benefits of implementing HBV NAT of blood donors is the reduction of the window period or time between infection and viral marker detection. Based on modeling of acute HBV infection, it is estimated that the HBsAg window period is approximately 25 to 42 days compared to approximately 21 to 28 days for HBV individual-donor NAT (ID-NAT), thereby reducing the already low risk of transfusion-transmitted HBV in Australia. It is also recognized that an additional benefit of HBV NAT is the detection of occult HBV infection (OBI), a form of chronic HBV infection characterized by undetectable HBsAg, usually low levels of HBV DNA and detectable anti-HBc. OBI in blood donors has now been widely reported and the phenomenon comprehensively reviewed. The risk associated with blood components from donors with OBI in Australia was recently estimated as approximately 1 in 982,000 per unit transfused, constituting 55% of the total HBV residual risk, which is estimated at approximately 1 in 538,000 per unit transfused. Lookback studies have confirmed that blood components from donors with OBI can transmit HBV, although, depending on the component type and the recipient’s immune status, transmission frequency may not be as high as that observed for components from donors in the pre-HBsAg window period.

In this report we present the results for the first 2 years of HBV ID-NAT of Australian blood donors and provide the first report of OBI in the Australian donor population. In particular, we compare the results for the first and second years of screening and highlight the potential complexity of detecting and confirming OBI.

MATERIALS AND METHODS

Donors

The Blood Service is responsible for the collection, testing, and processing of all allogeneic blood donations in Australia. All donations are from voluntary donors and, during the study period, were tested at one of five testing sites located in the major cities of Sydney, Melbourne, Brisbane, Perth, and Adelaide. Donors with evidence of HBV infection (as indicated by detection of HBV DNA and/or HBsAg) are notified of their results and called back for appropriate postdonation follow-up. At callback, a sample is taken for retesting. All donations screened for HBV markers during the period from July 5, 2010 (commencement of donor screening by HBV NAT), to July 4, 2012, were included in this study.

HBV screening and confirmatory assays

All donations were tested for HBsAg using a HBsAg chemiluminescent immunoassay (ChLIA; Abbott PRISM, Abbott Diagnostics, Delkenheim, Germany) and screened individually for HIV-1 RNA, HCV RNA, and HBV DNA with a HIV-1, HCV, and HBV multiplex assay (PROCLEIX ULTRIO, Gen-Probe/Novartis Diagnostics, San Diego/Emeryville, CA) on an automated system (PROCLEIX TIGRIS, Gen-Probe/Novartis Diagnostics). The 95% limit of detection for HBV DNA using the Ultrio assay is reported by the manufacturer as 10.4 IU/mL. Universal anti-HBc screening of donations is not performed in Australia. Only donations reactive on the PRISM HBsAg ChLIA and/or the Ultrio assay, or those from donors reporting a history of hepatitis or jaundice, were tested for anti-HBc. Anti-HBc testing was performed by either the AxSYM CORE or Architect anti-HBc II assays, and anti-HBs testing by either the AxSYM AUSAB or Architect AUSAB assays (Abbott Diagnostics).

Samples initially reactive on the Ultrio assay were "discriminated" to identify the specific virus using the PROCLEIX HIV-1, HCV, and HBV discriminatory assays. Ultrio reactive samples that tested nonreactive on all three discriminatory assays were retested on the Ultrio assay and, regardless of the repeat Ultrio results, tested for anti-HBc and anti-HBs. Samples that tested nonreactive on the PRISM HBsAg ChLIA but reactive on the Ultrio and HBV discriminatory assays were also tested by an alternate HBV NAT assay (Roche COBAS Ampliscreen HBV assay, Roche Diagnostics, Indianapolis, IN) and for anti-HBc and anti-HBs. Viral load testing was performed using either the RealTime HBV assay (Abbott Diagnostics) or COBAS AmpliPrep/COBAS TaqMan HBV Test v2.0 (Roche Diagnostics). For callback samples the NAT protocol was to initially test samples on the HBV discriminatory assay and, only if nonreactive, further test on the Ultrio assay. However, in some instances, callback samples that tested reactive on the HBV discriminatory assay were also tested on the Ultrio assay.

Donor HBV status

Donors who tested nonreactive on the PRISM HBsAg ChLIA but reactive on the Ultrio and/or HBV discriminatory assays were assessed as follows.

- **Acute HBV serologic window period infection:** nonreactive on the PRISM HBsAg ChLIA, anti-HBc nonreactive, reactive on the Ultrio and HBV discriminatory assays, and confirmed by HBV DNA detection by alternate NAT assay and/or subsequent seroconversion.

- **OBI:** nonreactive on the PRISM HBsAg ChLIA, anti-HBc reactive and reactive on the Ultrio and HBV discriminatory assays on the index sample, or Ultrio reactive/HBV discriminatory assay nonreactive on index and callback samples.
• **HBV inconclusive:** nonreactive on the PRISM HBsAg ChLIA, anti-HBc reactive, reactive on the Ultrio assay but nonreactive on the HBV discriminatory assay on the index sample and subsequently anti-HBc reactive, and Ultrio and HBV discriminatory assays nonreactive on the callback sample.

### RESULTS

**Comparison of OBI and acute serologic window period detection rate between the first and second years of HBV NAT**

During the study period a total of 42 OBI cases were detected. The OBI detection rate significantly decreased ($p < 0.05$) during the second year compared to the first year of HBV NAT, primarily due to the significant decrease in the detection rate for repeat donors (Table 1, 5.42 vs. 2.82/100,000 donors). In contrast, the detection rate of acute serologic window period infections for all donors significantly increased during the second year of HBV NAT (0.36 vs. 1.09/100,000 donors), although the numbers were small.

**Intermittent detection of HBV DNA in OBI donors**

Of the 42 OBI cases, 10 (23.8%) were Ultrio reactive but HBV discriminatory assay nonreactive on the index sample and therefore initially assessed as HBV inconclusive, but were subsequently assessed as OBI cases after testing of the callback sample: five were HBV discriminatory assay reactive, four were HBV discriminatory assay nonreactive but Ultrio reactive, and one sample was nonreactive on both assays but HBV DNA was detected by an alternate NAT assay (Table 2). There were 32 OBI cases that were Ultrio reactive and HBV discriminatory assay reactive on the index donation of which 18 remained discriminatory HBV reactive at callback while 13 were discriminatory HBV nonreactive (five were Ultrio reactive and eight Ultrio nonreactive). One donor was not available for callback testing. Overall, 19 (45.2%) OBI cases gave one or more Ultriononreactive donations before detection (Table 3).

**Viral load and anti-HBs levels**

During the study period there were a total of 85 samples (42 index samples and 43 callback samples) from the 42 OBI donors. Viral load testing was not performed on all samples, usually due to insufficient sample volume. Viral load testing was performed on 28 of the 42 (66.6%) OBI donors, six on both the index and the callback samples, six on the index sample only, and 16 on the callback sample only. Of the 28 donors for whom viral load testing was performed, HBV DNA was not detected by the viral load assay in 11 (39.3%) and detected but below the level for quantification in eight (28.6%) donors. Of the remaining nine donors, seven had viral loads of not more than 66 IU/mL and two had viral loads of 226 and 250 IU/mL.

### TABLE 1. HBV NAT–yield donors: comparison of first 2 years of donor screening by HBV NAT

|                      | Year 1: July 5, 2010-July 4, 2011 | Year 2: July 5, 2011-July 4, 2012 | Total: July 5, 2010-July 4, 2012 |
|----------------------|----------------------------------|----------------------------------|----------------------------------|
| First-time donors    | (1,337,429 donations)            | (1,336,092 donations)            | (2,673,521 donations)            |
| Repeat donors        | (424,301)                        | (424,798)                        | (849,099)                        |
| Total donors         | (554,142)                        | (551,016)                        | (1,190,138)                      |

|                      | First-time donors                 | Repeat donors                    | Total donors                     |
|----------------------|----------------------------------|----------------------------------|----------------------------------|
| HBsAg +              | 4                                | 1                               | 5                                |
| Anti-HBc +           | 23                               | 4                               | 27                               |
| HBV DNA +            | 4                               | 1                               | 5                                |

|                      | First-time donors                 | Repeat donors                    | Total donors                     |
|----------------------|----------------------------------|----------------------------------|----------------------------------|
| Anti-HBc +           | 2                                | 0                               | 2                                |
| OBI detection rate   | 5.42                             | 2.82                            | 4.87                             |
| HBV discriminatory assay reactive rate | 1.54                           | 0.47                       | 1.06                             |

|                      | First-time donors                 | Repeat donors                    | Total donors                     |
|----------------------|----------------------------------|----------------------------------|----------------------------------|
| HBsAg +              | 4                                | 1                               | 5                                |
| Anti-HBc +           | 23                               | 4                               | 27                               |
| HBV DNA +            | 4                               | 1                               | 5                                |

|                      | First-time donors                 | Repeat donors                    | Total donors                     |
|----------------------|----------------------------------|----------------------------------|----------------------------------|
| Anti-HBc +           | 2                                | 0                               | 2                                |
| OBI detection rate   | 5.42                             | 2.82                            | 4.87                             |
| HBV discriminatory assay reactive rate | 1.54                           | 0.47                       | 1.06                             |

**TABLE 2. OBI donor results (n=42)**

| Donor test results | HBsAg | Anti-HBc |
|--------------------|-------|----------|
| First-time donors  |       |          |
| Repeat donors      |       |          |
| Total donors       |       |          |

|                      | First-time donors                 | Repeat donors                    | Total donors                     |
|----------------------|----------------------------------|----------------------------------|----------------------------------|
| Anti-HBc +           | 2                                | 0                               | 2                                |
| HBV discriminatory assay reactive rate | 5.42                           | 2.82                       | 4.87                             |

|                      | First-time donors                 | Repeat donors                    | Total donors                     |
|----------------------|----------------------------------|----------------------------------|----------------------------------|
| Anti-HBc +           | 2                                | 0                               | 2                                |
| OBI detection rate   | 5.42                             | 2.82                            | 4.87                             |
| HBV discriminatory assay reactive rate | 1.54                           | 0.47                       | 1.06                             |

|                      | First-time donors                 | Repeat donors                    | Total donors                     |
|----------------------|----------------------------------|----------------------------------|----------------------------------|
| Anti-HBc +           | 2                                | 0                               | 2                                |
| OBI detection rate   | 5.42                             | 2.82                            | 4.87                             |
| HBV discriminatory assay reactive rate | 1.54                           | 0.47                       | 1.06                             |

|                      | First-time donors                 | Repeat donors                    | Total donors                     |
|----------------------|----------------------------------|----------------------------------|----------------------------------|
| Anti-HBc +           | 2                                | 0                               | 2                                |
| OBI detection rate   | 5.42                             | 2.82                            | 4.87                             |
| HBV discriminatory assay reactive rate | 1.54                           | 0.47                       | 1.06                             |

**TABLE 3. OBI donor results (n=42)**

|                      | First-time donors                 | Repeat donors                    | Total donors                     |
|----------------------|----------------------------------|----------------------------------|----------------------------------|
| Anti-HBc +           | 2                                | 0                               | 2                                |
| OBI detection rate   | 5.42                             | 2.82                            | 4.87                             |
| HBV discriminatory assay reactive rate | 1.54                           | 0.47                       | 1.06                             |

**TABLE 4. OBI donor results (n=42)**

|                      | First-time donors                 | Repeat donors                    | Total donors                     |
|----------------------|----------------------------------|----------------------------------|----------------------------------|
| Anti-HBc +           | 2                                | 0                               | 2                                |
| OBI detection rate   | 5.42                             | 2.82                            | 4.87                             |
| HBV discriminatory assay reactive rate | 1.54                           | 0.47                       | 1.06                             |
respectively. Of the 42 OBI donors, 27 (64.3%) had anti-HBs levels of not more than 10 IU/L at their index donation while the remaining 15 (35.7%) had levels between 10 and 270 IU/L.

HBV inconclusive donors

During the study period 73 donors were initially classified as HBV inconclusive at the index sample and 68 were available for callback. Of these 68, 10 (14.7%) were reclassified as OBI cases, seven (10.3%) were nonreactive for both anti-HBc and NAT at callback and therefore

| TABLE 2. Summary of HBV NAT results for OBI donors: index and callback samples |
|---------------------------------------------------------------|
| OBI case number | Previous Ultrio-nonreactive donations | Index sample | Callback sample |
|-----------------|----------------------------------------|--------------|-----------------|
|                 |                                        | Ultrio | dHBV | Alternate HBV NAT† | Ultrio | dHBV |
| 1               | 0                                      | +     | +    | +                | -      | +    |
| 2               | 0                                      | +     | +    | -                | +      | -    |
| 3               | 0                                      | +     | +    | -                | +      | NT   |
| 4               | 0                                      | +     | +    | +/-              | NT     | +    |
| 5               | 0                                      | +     | +    | +                | NT     | +    |
| 6               | 0                                      | +     | +    | +                | -      | -    |
| 7               | 0                                      | +     | +    | +                | NT     | +    |
| 8               | 0                                      | +     | +    | +                | NT     | +    |
| 9               | 2                                      | +     | +    | -                | NT     | +    |
| 10              | 0                                      | +     | +    | +                | -      | +    |
| 11              | 0                                      | +     | +    | +                | -      | -    |
| 12‡             | 0                                      | +     | +    | +                | NT     | +    |
| 13              | 0                                      | +     | +    | +                | NT     | +    |
| 14              | 0                                      | +     | +    | -                | NT     | +    |
| 15              | 0                                      | +     | +    | NT               | NT     | +    |
| 16              | 0                                      | +     | +    | -                | NT     | +    |
| 17              | 0                                      | +     | +    | NT               | NT     | +    |
| 18              | 1                                      | +     | +    | +/-              | NT     | +    |
| 19              | 2                                      | +     | +    | -                | NT     | +    |
| 20              | +                                      | +     | +/-       | NT               | -      | +    |
| 21              | 2                                      | +     | +/-       | NT               | -      | +    |
| 22§             | 0                                      | +     | -      | NT               | -      | +    |
| 23‡             | 2                                      | +     | -      | NT               | -      | +    |
| 24              | 3                                      | +     | +      | -                | NT     | +    |
| 25              | 3                                      | +     | +      | +/-              | +      | -    |
| 26              | 3                                      | +     | +      | NT               | -      | -    |
| 27‡             | 0                                      | +     | -      | NT               | +      | -    |
| 28              | 0                                      | +     | +      | NT               | +      | -    |
| 29‡             | 4                                      | +     | -      | NT               | NT     | +    |
| 30§             | 0                                      | +     | +      | NT               | NT     | +    |
| 31              | 0                                      | +     | +      | NT               | NT     | +    |
| 32§             | 33                                    | +      | -     | NT               | NT     | +    |
| 33              | 3                                      | +     | +      | +/-              | NT     | NT   |
| 34              | 1                                      | +     | +      | +                | +      | +    |
| 35‡             | 3                                      | +     | -      | -                | +      | -    |
| 36              | 3                                      | +     | +      | +                | -      | -    |
| 37              | 4                                      | +     | -      | NT               | -      | -    |
| 38§||           | 0                                      | +     | -      | NT               | -      | -    |
| 39              | 8                                      | +     | +      | +                | -      | -    |
| 40‡             | 6                                      | +     | -      | -                | NT     | +    |
| 41              | 6                                      | +     | +      | NT               | -      | -    |
| 42              | 1                                      | +     | +      | -                | -      | -    |

* OBI (see text for details); all OBI cases were HBsAg− and anti-HBc+.
† Cobas Ampliscreen HBV assay, Roche Diagnostics.
‡ Index donation was reactive on Ultrio, nonreactive on HBV discriminatory, and nonreactive when retested on Ultrio.
§ Index donation was reactive on Ultrio, nonreactive on HBV discriminatory, and reactive when retested on Ultrio.
|| HBV DNA detected on callback sample by alternate NAT assay.
NT = not tested; + = reactive; − = nonreactive.

| TABLE 3. OBI donors: Ultrio-nonreactive donations before index-reactive result |
|---------------------------------------------------------------|
| Number of previous Ultrio-nonreactive donations before index-reactive result | Number of cases† |
| Number of cases† | July 5, 2010- | July 5, 2011- | July 4, 2011 | July 4, 2012 |
|-------------------|---------------|---------------|--------------|--------------|
| 0                 | 19 (70.4)     | 4 (26.7)      |              |              |
| 1-3               | 8 (29.6)      | 5 (33.3)      |              |              |
| 4-8               | 0             | 6 (40.0)      |              |              |
| Total             | 27 (100.0)    | 15 (100.0)    |              |              |

* See text for details.
† Data are reported as number (%).
DISCUSSION

HBV NAT and OBI detection

This study represents the first report on the screening of Australian blood donors by HBV NAT. During the first 2 years of HBV NAT, a total of 2,673,521 donations were tested, representing 756,915 donors. We detected eight acute HBV serologic window period infections in contrast to 42 OBI cases. Most of these 42 donors identified as OBI cases were repeat donors, which is consistent with existing infection; it is unusual to detect new HBV infections among repeat donors in Australia. These results reflect the existence of a subset of repeat donors with OBI within the Australian donor population before the advent HBV NAT and highlight two important issues regarding the detection of OBI in jurisdictions without universal anti-HBc screening. First, most OBI cases detected in the initial period subsequent to the implementation of HBV NAT will be repeat donors and over time the OBI detection rate would be expected to decline with new donors representing an increasing proportion of cases. Second, the implementation of HBV NAT in jurisdictions without universal anti-HBc screening will initially result in a high proportion of OBI cases being detected the first time they are tested, but this proportion will decrease over time. This was highlighted in our study by the observation that during the first year of HBV NAT screening the percentage of OBI donors detected the first time they were tested by HBV NAT was 70.4% compared to 26.7% during the second year. This can be explained by the detection, during the first year of HBV NAT, of those OBI donors with relatively higher levels of HBV DNA and who therefore have a greater probability of detection the first time they are tested. In the absence of universal anti-HBc screening of donors, the Blood Service has employed a “history of hepatitis” algorithm according to which donors who report a history of hepatitis or jaundice are tested for anti-HBc. If reactive, donors are deferred unless their anti-HBs level is at least 100 IU/L. Before the implementation of HBV NAT, this algorithm of selective anti-HBc testing would have interdicted the majority of OBI cases and require follow-up testing.

Confirming OBI

A number of observations from our study demonstrate that the confirmation of OBI can be difficult, particularly for those OBI cases with very low HBV DNA levels. First, as already noted, HBV DNA may only be intermittently detectable over time, even by ID-NAT. Thirty-two of the 42 OBI cases were reactive on the Ultro and HBV discriminatory assays at the index donation and eight of these 32 were nonreactive on both assays at callback. This intermittent detectability of OBI by HBV NAT was recently demonstrated in a South African study in which samples from 57 low-viral-load OBI cases were retrospectively tested in replicates of 6 (a total of 342 tests) on three different NAT assays: Ultro, Ultro Plus, and Taqscreen. The overall detection rates for these 342 tests were 52 and 73% for ID-Ulтро and ID-Ulтро Plus, respectively, and 39% for Taqscreen (MP6). A second factor that may complicate the confirmation of OBI is discordant results between multiplex and discriminatory assays. In our study, 10 of the 42 OBI cases were Ulтро reactive but HBV discriminatory assay nonreactive at the index donation. Similarly, of the 43 callback samples, 26 were tested on both the Ulтро and HBV discriminatory assays and 13 gave discordant results (four were Ulтро nonreactive/HBV discriminatory reactive and nine were Ulтро reactive/HBV discriminatory nonreactive). This finding of discordant results between Ulтро multiplex and discriminatory assays for the same OBI donor sample has also been reported by others.

Further, our results highlight the need for caution and follow-up testing of donors assessed as HBV inconclusive (anti-HBc reactive/Ulтро reactive/HBV discriminatory assay nonreactive) on the index sample. Of 73 such donors, 68 were available for callback and 10 were confirmed as OBI on the callback sample (Ulтро and/or HBV discriminatory reactive or alternate NAT assay reactive) some 2 to 8 weeks after their respective index donations. The importance of this cautious approach is further indicated by two recent studies. A New Zealand study reported that donors with NAT nonrepeatable–reactive results on either the Ulтро or the Ulтро Plus assay (defined as reactivity on a multiplex assay but no reactivity on all three discriminatory assays) had a significantly higher anti-HBc reactive rate compared to the general donor population. Similarly, a South African study reported a higher prevalence of anti-HBc in donors who initially tested Ulтро reactive but nonreactive when retested in duplicate (Ulтро non–repeat reactive) compared to donors who initially tested Ulтро nonreactive (12.2% vs. 6.6%, respectively). These observations provide further evidence that a proportion of donors who are anti-HBc reactive, Ulтро reactive, and HBV discriminatory nonreactive represent OBI cases and require follow-up testing.

Residual risk due to OBI donors

The potential clinical implications of donors with OBI were recently highlighted by a Japanese report of a case of transfusion-transmitted HBV from an OBI donor who, at the time of the implicated donation, was anti-HBc reactive with an anti-HBs level of 29.6 IU/L but without detectable
HBsAg or HBV DNA. In addition, a recent European study indicated that products from OBI donors may have an overall transmission rate of 48% with rates varying between different components—24% for red blood cells, 51% for platelets, and 85% for fresh frozen plasma. The proportionate residual risk associated with window period versus OBI donors is dependent on the testing strategy employed. For countries with universal anti-HBc testing the OBI risk is negligible since virtually all donors with OBI will have detectable anti-HBc and thus will be interdicted. However, for countries like Australia that lack universal anti-HBc testing, the relative risk of OBI is substantially increased. We recently reported a novel method to calculate the OBI risk component that estimated the OBI risk in the Australian donor population was approximately 1 in 982,000 per unit transfused. This OBI risk represented the majority (55%) of the total HBV residual risk, which was estimated at approximately 1 in 538,000 per unit transfused based on HBV testing data from January 2012 to March 2013. The identification and referral for clinical assessment of 42 previously undetectable HBV infected donors in the first 2 years of HBV NAT screening clearly establishes a declining risk.

Study limitation
The primary limitation of our study was the use of the Ultrio assay. It is acknowledged that the use of the Ultrio Plus assay, which is more sensitive for HBV DNA detection than the Ultrio assay, may have resulted in the detection of more OBI cases and clarified the HBV status of some of the donors who were HBV inconclusive at the index donation but tested anti-HBc reactive and NAT non-reactive at callback. Although the Ultrio Plus assay has recently been implemented by the Blood Service, we believe that the use of the less sensitive Ultrio assay may have been in part compensated for by our history of hepatitis algorithm and cautious approach to managing HBV inconclusive donors.

In summary, we have provided the first report of OBI among Australian blood donors based on the first 2 years of HBV ID-NAT. Our results demonstrate the value of HBV ID-NAT for the detection of both acute serologic window period and OBI donors in a HBV low-prevalence country without universal anti-HBc screening. In our donor population we found a substantially higher prevalence of OBI cases compared to acute HBV serologic window period infections. For samples reactive on the Ultrio assay but nonreactive on the HBV discriminatory assay, the inclusion of anti-HBc in our testing algorithm is pivotal in identifying OBI donors with intermittently detectable HBV DNA. The incremental identification and deferral of OBI donors, along with the detection of acute serologic window period donors, will further reduce the residual risk of HBV infection in Australia, which is currently estimated to be approximately 1 in 538,000. However, further refinement to the risk modeling to more precisely incorporate the risk from OBI donors with viral loads below the limit of detection of ID NAT is a priority as our study demonstrates that OBI can be difficult to detect and confirm.

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CONFLICT OF INTEREST
The authors report no conflicts of interest or funding sources.

REFERENCES
1. Seed CR, Margaritis AR, Bolton WV, et al. Improved efficiency of national HIV, HCV, and HTLV antibody testing algorithms based on sequential screening immunoassays. Transfusion 2003;43:226-34.
2. Mison L, Seed CR, Margaritis AR, et al. Nucleic acid technology screening of Australian blood donors for hepatitis C and human immunodeficiency virus-1 RNA: comparison of two high-throughput testing strategies. Vox Sang 2003;84:11-9.
3. Margaritis AR, Brown SM, Seed CR, et al. Comparison of two automated nucleic acid testing systems for simultaneous detection of human immunodeficiency virus and hepatitis C virus RNA and hepatitis B virus DNA. Transfusion 2007;47:1783-93.
4. Ismay SL, Thomas S, Fellows A, et al. Post-transfusion hepatitis revisited. Med J Aust 1995;163:74-7.
5. Seed CR, Lee JF, Maloney R, et al. Hepatitis B surface antigenemia in blood donors after vaccination. Transfusion 1996;36:386.
6. Kleinman SH, Busch MP. HBV: amplified and back in the blood safety spotlight. Transfusion 2001;41:1081-5.
7. Kleinman SH, Busch MP. Assessing the impact of HBV NAT on window period reduction and residual risk. J Clin Virol 2006;36(Suppl 1):S23-9.
8. Seed CR, Kiely P, Keller AJ. Residual risk of transfusion transmitted human immunodeficiency virus, hepatitis B virus, hepatitis C virus and human T lymphotrophic virus. Intern Med J 2005;35:592-8.
9. Biswas R, Tabor E, Hsia CC, et al. Comparative sensitivity of HBV NATs and HBsAg assays for detection of acute HBV infection. Transfusion 2003;43:788-98.
10. Busch MP, Glynn SA, Stramer SL, et al. A new strategy for estimating risks of transfusion-transmitted viral infections based on rates of detection of recently infected donors. Transfusion 2005;45:254-64.

11. Assal A, Barlet V, Deschaseaux M, et al. Sensitivity of two hepatitis B virus, hepatitis C virus (HCV), and human immunodefi ciency virus (HIV) nucleic acid test systems relative to hepatitis B surface antigen, anti-HCV, anti-HIV, and p24/anti-HIV combination assays in seroconversion panels. Transfusion 2009;49:301-10.

12. Candotti D, Allain JP. Transfusion-transmitted hepatitis B virus infection. J Hepatol 2009;51:798-809.

13. Hollinger FB. Hepatitis B virus infection and transfusion medicine: science and the occult. Transfusion 2008;48:1001-26.

14. Allain JP. Occult hepatitis B virus infection: implications in transfusion. Vox Sang 2004;86:83-91.

15. Stolz M, Tinguey C, Graziani M, et al. Efi cacy of individual nucleic acid amplifi cation testing in reducing the risk of transfusion-transmitted hepatitis B virus infection in Switzerland, a low-endemic region. Transfusion 2010;50:2695-706.

16. Manzini P, Girotto M, Borsotti R, et al. Italian blood donors with anti-HBc and occult hepatitis B virus infection. Haematologica 2007;92:1664-70.

17. O’Brien SF, Fearon MA, Yi QL, et al. Hepatitis B virus DNA-positive, hepatitis B surface antigen-negative blood donations intercepted by anti-hepatitis B core antigen testing: the Canadian Blood Services experience. Transfusion 2007;47:1809-15.

18. Candotti D, Grabarczyk P, Ghiazza P, et al. Characterization of occult hepatitis B virus from blood donors carrying genotype A2 or genotype D strains. J Hepatol 2008;49:537-47.

19. Allain JP, Belkhir D, Vermeulen M, et al. Characterization of occult hepatitis B virus strains in South African blood donors. Hepatology 2009;49:1868-76.

20. Katsoulidou A, Paraskevis D, Magjorkinis E, et al. Molecular characterization of occult hepatitis B cases in Greek blood donors. J Med Virol 2009;81:815-25.

21. Vermeulen M, Lelie N, Sykes W, et al. Impact of individual-donation nucleic acid testing on risk of human immunodeficiency virus, hepatitis B virus, and hepatitis C virus transmission by blood transfusion in South Africa. Transfusion 2009;49:1115-25.

22. Gonzalez R, Torres P, Castro E, et al. Efi cacy of hepatitis B virus (HBV) DNA screening and characterization of acute and occult HBV infections among blood donors from Madrid, Spain. Transfusion 2010;50:221-30.

23. Altunay H, Kosan E, Birinci I, et al. Are isolated anti-HBc blood donors in high risk group? The detection of HBV DNA in isolated anti-HBc cases with nucleic acid amplifi cation test (NAT) based on transcription-mediated amplifi cation (TMA) and HBV discrimination. Transfus Apher Sci 2010;43:265-8.

24. Panigrahi R, Biswas A, Datta S, et al. Anti-hepatitis B core antigen testing with detection and characterization of occult hepatitis B virus by an in-house nucleic acid testing among blood donors in Behrampur, Ganjam, Orissa in southeastern India: implications for transfusion. Virol J 2010;7:204.

25. Asim M, Ali R, Khan LA, et al. Signifi cance of anti-HBc screening of blood donors & its association with occult hepatitis B virus infection: implications for blood transfusion. Indian J Med Res 2010;132:312-7.

26. Liu Y, Li P, Li C, et al. Detection of hepatitis B virus DNA among accepted blood donors in Nanjing, China. Virol J 2010;7:193.

27. Louisirirotchanakul S, Oota S, Khuponsarb K, et al. Occult hepatitis B virus infection in Thai blood donors. Transfusion 2011;51:1532-40.

28. Seo DH, Whang DH, Song EY, et al. Prevalence of antibodies to hepatitis B core antigen and occult hepatitis B virus infections in Korean blood donors. Transfusion 2011;51:1840-6.

29. Kleinman SH, Lelie N, Busch MP. Infectivity of human immunodeficiency virus-1, hepatitis C virus, and hepatitis B virus and risk of transmission by transfusion. Transfusion 2009;49:2454-89.

30. Seed CR, Kiely P. A method for estimating the residual risk of transfusion-transmitted HBV infection associated with occult hepatitis B virus infection in a donor population without universal anti-HBc screening. Vox Sang 2013;105:290-8.

31. Satake M, Taira R, Yugi H, et al. Infectivity of blood components with low hepatitis B virus DNA levels identifi ed in a lookback program. Transfusion 2007;47:1197-205.

32. Yuen MF, Wong DK, Lee CK, et al. Transmissibility of hepatitis B virus (HBV) infection through blood transfusion from blood donors with occult HBV infection. Clin Infect Dis 2011;52:624-32.

33. Su TH, Chen PJ, Chen TC, et al. The clinical signifi cance of occult hepatitis B transfusion in Taiwan—a look-back study. Transfus Med 2011;21:33-41.

34. Hanada D, Kino S, Yamauchi S, et al. Transfusion-transmission of hepatitis B virus (HBV) from a NAT-negative occult hepatitis B virus carrier. Vox Sang 2011;100:129-34.

35. Allain JP, Mihaljevic I, Gonzalez-Fraile MI, et al. Infectivity of blood products from donors with occult hepatitis B virus infection. Transfusion 2013;53:1405-15.

36. Polizzotto MN, Wood EM, Ingham H, et al. Reducing the risk of transfusion-transmissible viral infection through blood donor selection: the Australian experience 2000 through 2006. Transfusion 2008;48:55-63.

37. Vermeulen M, Coleman C, Mitchell J, et al. Sensitivity of individual-donation and minipool nucleic acid amplifi cation test options in detecting window period and occult hepatitis B virus infections. Transfusion 2013;53(Suppl 3):2459-66.
38. Brojer E, Grabarczyk P, Liszewski G, et al. Characterization of HBV DNA+/HBsAg- blood donors in Poland identified by triplex NAT. Hepatology 2006;44:1666-74.
39. Yang MH, Li L, Hung YS, et al. The efficacy of individual-donation and minipool testing to detect low-level hepatitis B virus DNA in Taiwan. Transfusion 2010;50:65-74.
40. Charlewood R, Flanagan P. NAT non-repeatable reactivity and HBV infection. Vox Sang 2011;101:13.
41. Cable R, Lelie N, Bird A. Reduction of the risk of transfusion-transmitted viral infection by nucleic acid amplification testing in the Western Cape of South Africa: a 5-year review. Vox Sang 2013;104:93-9.
42. Tsoi WC, Lelie N, Lin CK. Enhanced detection of hepatitis B virus in Hong Kong blood donors after introduction of a more sensitive transcription-mediated amplification assay. Transfusion 2013;53(Suppl 3):2477-88.
43. Stramer SL, Notari EP, Krysztof DE, et al. Hepatitis B virus testing by minipool nucleic acid testing: does it improve blood safety? Transfusion 2013;53(Suppl 3):2449-58.