Title: Nearly half of Ultrio Plus NAT non-discriminated reactive blood donors were identified as occult HBV infection in south China

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Reviewer: Syria Laperche

Reviewer's report:

This study presents the results of investigations performed in Shenzhen blood setting in HBV/HCV/HIV multiplex NAT initially reactive blood donations, seronegative for markers included in the routine screening (HBsAg, HIVAb and HCV Ab) which resulted negative when further tested with discriminatory NAT assays (n=259).

Authors show that 91% (236/259) of these NDR donations were anti-HBc positive and 47% (121/259) had HBV DNA when tested with alternative amplification methods (classified as OBI).

On remaining 53% (n=138), 89% (123) had anti-HBc alone or associated with HBsAb

This study is certainly helpful to adopt an adapted strategy to avoid HBV transmission by transfusion from OBI donations since anti-HBc cannot be systematically tested in China due to the high HBV infection prevalence and helps to define a reliable algorithm to permit non-infected donors to come back to donate.

General comments:
- The paper is difficult to read especially because results are presented several times in different ways ex: anti HBc positive are detailed in section 3.2 then 3.3 ; anti HBs in section 3.2 and 3.4... The presentation according the flow showed in figure1 should clarify the text and lead to a better understanding.
- 53% of NDR were inconclusive as HBV DNA was not detected. Although nearly 90% of these samples were anti HBc pos, suggesting that HBV infection could be the cause of them, HIV or HCV early infections with low viral loads cannot be totally ruled out. Donor follow up (not performed) would have helped to conclude.
- Except the 121 NDR donors who have been confirmed HBV DNA positive by alternative PCRs, no confirmation of NDR samples has been presented: were Ultrio Plus initially positive repeated?
- Were antiHBc positive results confirmed?
- Were presence of mutations confirmed with another sequencing?
- Authors showed that 91% of NDR donations were antiHBc positive, is this rate the same in blood donors negative for all viral markers?
- 121 donors have been classified as OBI : were the VL determined?
- Authors did not clearly claim whether observed mutations are responsible for OBI.
- Follow up results to confirm OBI should have been useful
  – Many results have been repeated in the discussion I suggest making an effort to avoid redundancies in order to reduce the discussion by focusing on the key results, suggestions for adopting better donor and donation strategies and limitations of the study.

Specific comments
- Introduction: refer to Candotti et al GUT 2009 to show that OBI are potentially at risk in transfused patients
- Line 107: please mention if IR samples with multiplex NAT were repeated
- Line 114: HBeAg and HBeAb results were not provided
- Line 118: give the LoD of qPCR. Why VLs were not provided? Clearly clarify which samples have been tested with qPCR?
- Line 138: 215 samples were HBsAg pos only. This is a surprisingly high number, knowing (i) the expected high rate of chronic carriers who should have been NAT pos and/or HBcAb pos and (ii) the high sensitivity of NAT. Have these samples been confirmed HBsAg pos?
- Lines 161-169: difficult to understand "68 donations of 82 qPCRpos" 82 sequences: I assume that some samples have been amplified in 2 regions? Please clarify
- Line 172: Have the 2 donors classified as WP been followed up be to attest that they were early infections?
- Lines 179-190: were anti-HBs pos donors vaccinated?
- Lines 211-232: were strains with S mutations affecting HBs detection controlled with an alternative HBsAg assay? If positive they must be excluded from OBI classification
- Tables 1-2 to be merged

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Unable to assess

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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Not relevant to this manuscript

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