Reviewer’s report

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

**Version:** 0  **Date:** 01 Feb 2019

**Reviewer:** Jean-Pierre Allain

Reviewer's report:

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The manuscript by Ye et al reports on the difficulties introduced by a NAT triplex manufacturer with a two-step assay first with a ubiquitous signal followed by a specific discriminatory assay less sensitive than the triplex. This topic has been examined by several previous publications including two from China. It is therefore not novel and the data presented essentially confirm prior results.

The main problem with this manuscript is that although the data appears solid, the presentation is not logically constructed and the order of text and tables makes the data unclear and difficult to follow.

1. In Materials and Methods, it should be made clear that the third HBsAg assay used to qualify NDRs is different from the ELISA used in screening.

The authors should include a section regarding 'confirmation' of initial reactive NAT. Is it Ultrio Plus and either qPCR or NPCR positive? This reviewer considers it is confirmatory but, the importance of sensitivity of both confirmatory assays should be clearly established by indicating what amount of the 2.5ml extract is used in each assay relative to the 0.5ml of Ultrio+ and what is the limit of detection of these 2 confirmatory assays.

In the DNA sequencing section, the size of each amplicon should be given. It should also be mentioned that for genotyping only S sequences are informative.

There is no section regarding ethical approval.

2. In the result section, Table 1 should be presentation of serologic data currently as Table 3. The data currently in Table 4 regarding titration of anti-HBs should equally be part of that section as Table 2. Table 1 should present a complete picture of the serological markers versus NAT confirmatory including all 421 samples, stratified into confirmed HBV DNA positive and negative (not confirmed). The section 3.3 should be divided into 2 subsection: 1) confirmatory with Table 3 (currently Table 2)
and 2) overall data related to sequencing. The latter should present in Table 4 (currently Table 1) samples with both S and BCP/PC sequences, BCP/PC only and S only. It might be then useful to add in this table the number of sequences in each group that presented 1727 mutations, 1762-1764 mutations and most importantly 1896 mutations and combinations of mutations in those 3 regions. In that respect, it would be useful to indicate which 1896 mutated samples were anti-HBe positive.

Section currently 3.5 should also be divided into 2 subsections 1) genotyping and 2) MHR or outside of MHR mutations. In this section, the authors take a group of mutations as 'vaccine related' without justifying their choice by references. To my knowledge, only the G145 aa is clearly related to vaccination and appears in this data as the most frequent substitution. A second group of substitutions should be those demonstrated as interfering with HBsAg assays (supported by adequate references). In that respect, the authors did not mention that contrary to many reports on OBI, their sequences did not include mutations of P120 or any C 121, 124, 137, 139, 147, 149 which are shown to have a major impact on HBsAg detection. They did not either find substitutions at aa 75, 100 or 178, shown responsible in OBIs for lack of excretion of HbsAg.

3. The discussion should be structured around key questions raised by this and previously published data on the same topic:

1. Critical importance of design and sensitivity of screening assays. Increased sensitivity with the 2-stage assay carries increasing difficulties in confirmation (13% with Ultrio and 57% of NDR with Ultrio Plus in New Zealand). Note that this problem has been eliminated by the latest Roche assay directly identifying viruses.
2. Algorithm for confirmation. There are several publications advocating repeat testing with the screening assay from the plasma bag (not addressed). Alternative assay as done here is another approach highly dependent on respective sensitivity of assays and choice of regions amplified. Pros and cons should be discussed.
3. Usefulness of serology. Here anti-HBc is not helpful contrary to other publications from China and from other parts of the world. This 'anomaly' requires discussion.
4. What to do with and tell the donors? The authors do not consider in their analysis that serology negative may well be 'primary OBI' rather than window period.
5. Implications for transfusion risk. It was clearly shown (ref 21) that Ultrio Plus negative donations can be infectious. Therefore NDR not confirmed but anti-HBc positive might be a safety risk. Only detection of anti-HBs >100IU/L appears protective.

In conclusion, this manuscript needs to be entirely re-written to make good data palatable to the readers.

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

No

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.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

Not relevant to this manuscript

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Needs some language corrections before being published

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