Author's response to reviews

Title: Evaluating HBsAg rapid tests performance for different biological samples from low- and high infection rate settings & populations

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Author's response to reviews: see over
Thank you for your review of the manuscript titled “Evaluating HBsAg rapid test performance for different biological samples from low and high infection rate settings & populations” by Helena M Cruz, Leticia P Scalioni, Vanessa S de Paula, Elisângela F da Silva, Kycia Maria R do Ó, Flavio AP Milagres, Marcelo S Cruz, Francisco I Bastos, Priscila Pollo-Flores, Erotildes Leal, Ana Rita C Motta-Castro, José Henrique Pilotto, Lia Laura Lewis-Ximenez, Elisabeth Lampe and Livia M Villar. We appreciate your efforts and those of the reviewers.

We are in agreement with the observations made by the reviewers, and the changes made in response have improved our manuscript. These modifications are shown in the text in red color.

I am forwarding our responses to the questions and comments that have resulted in revisions of the manuscript.

Sincerely,

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Editorial request:

1. Abbreviations:

Please include a list of abbreviations used in the manuscript and their meanings after the Conclusions section.

This section is now included after the conclusion section.

Reviewer: SIMON François

Major comments:
- Are the 3 RDT FDA approved and/or CE IVD? With a WHO registration? This a major information for the publication in an international peer review

The following was added to the methods section, Rapid tests evaluation section, lines 189 to 194: “All the RTs are approved by the Brazilian National Health Surveillance Agency (ANVISA), which is responsible for the regulation, control and supervision of products and services that involve risk to public health. Vikia® HBsAg has CE IVD approval but does not have FDA approval or WHO registration. Imuno-Rápido HBsAg® does not have FDA approval or WHO registration; such information is not available for HBsAg Teste Rápido®.”

- Identify always the test with the same designation as Vikia HBsAg, HBsAg Teste Rápido or Imuno-Rápido HBsAg all along the paper. Better than by their manufacturers (BioMérieux, Doles or Wama) and better than the present mix

We agree with this observation, and the same designation of Vikia HBsAg, HBsAg Teste Rápido or Imuno-Rápido HBsAg was used throughout the paper.

- OF/ Saliva results are poorly informative and complicates the presentation. These samples were probably collected during previous HCV study for HCV and OF data brings little to the article. Could be deleted

Saliva samples were collected for the evaluation of rapid tests for HBV. As the RTs were not developed for saliva samples, the sample volume was increased. Indeed, the results obtained were not satisfactory, but this information could be helpful for demonstrating that these assays could not be used to detect HBsAg in saliva samples.

- Discussion must take account of the recent published meta-analyzes on this HBs RDT topic and reference sections should included ie Shivkumar S et al Rapid point-of-care first-line screening tests for hepatitis B infection: a meta-analysis of diagnostic accuracy (1980-2010)


These references are included in the reference section (numbers 20 and 21) as well as the discussion section (lines 369-375).
Reviewer: Edouard Tuaillon

I. Major Compulsory Revisions:
1. The populations tested for HBV infection are clearly described. For example the difference between the reference panel and the group I is not clearly explained. Sentence like “Most of them lived in underserved and impoverished areas in the Rio de Janeiro State” appears approximate. Some of the individuals of the group II lived in the Rio de Janeiro state. Hence, inclusion criteria need to be simplified and clarified in the text. A map could be included as a new figure to shown location of each group, number of samples, time of collection, population characteristics (general population, poor resource area, high risk group).

The inclusion criteria have been clarified in the text. The reference panel comprised serum samples obtained from individuals recruited at Viral Hepatitis Ambulatory. The inclusion criteria for this group were as follows: acute, chronic or suspected viral hepatitis; age more than 18 years; and signed informed consent.

Group I was composed of individuals recruited at Viral Hepatitis Ambulatory who provided serum, whole blood and saliva samples. Serum samples were tested for HBsAg using one ELISA kit (ETI-MAK-4, Diasorin, Italy). In this setting, the inclusion criteria was attendance at Fiocruz Viral Hepatitis Ambulatory, residing in underserved and impoverished areas in Rio de Janeiro State and a suspected case of viral hepatitis infection.

Individuals from the general population of two cities of Rio de Janeiro State were included as Group II. Petrópolis city is located in a mountainous region, and Macaé city is situated in North Rio de Janeiro State. None of these individuals were recruited in viral hepatitis ambulatory care settings, and this group was considered to be a low risk for HBV.
This information was added to the methods, study population section.

2. Authors have written that serological characteristics of the sera are available: “Sera samples (positive for HBsAg) were also assayed for total anti-HBc, IgM anti-HBc, anti-HBs, HBeAg and anti-HBe using commercial EIAs (Diasorin, Italy)”, page 8 line 172. However this important data are not shown. These results should be shown in a table and discussed.
We agree with this recommendation. The results of these markers are included in a table (Appendix 2) and presented in the results and discussion sections.

3. Discordant results EIA +/rapid test – need to be characterized. DO value of the EIA were low, do the authors are sure that sera were true positive for HBV? In these cases, further analyses are request: AgHBs neutralization assays, and HBV DNA testing, HBsAg concentration. If this data are not available this major limit should be discussed.
To confirm the HBsAg results, all reactive HBsAg serum samples were retested in duplicate, and only concordant results were included in the study. Indeed, AgHBs neutralization assays, HBV DNA testing, and the HBsAg concentration were not determined. This limitation was included in the discussion section, as suggested (line 392-394).
II. Minor Essential Revisions:
- The analytical sensitivity (ex less than or equal to 2 IU/ml (3.8 ng/ml for the Vikia assay based on the manufacturer technical manual) need to be indicated in the method section. It would especially interesting to confirm and compare the analytical sensitivity for each of the tests using dilution the WHO standard as a first result of the study. Possibility to test capillary blood should also be indicated in the text. The analytical sensitivity was included in the methods section, rapid test evaluation section, lines 200-202. The possibility of using capillary blood is now indicated in the text in the Rapid test evaluation section, lines 198-199.

- The following sentence should not be included in this methods section “From 1999 to 2011, 120,343 confirmed HBV cases were reported in Brazil, most of them in the Southeast region (36.3%), where Rio de Janeiro is located (10)”, but in the discussion section. The sentence was included in the discussion section, as suggested (lines 337-339).

- The study of Helena M Cruz et al demonstrates that HBsAg testing on saliva using these three rapid tests had low performances. The authors should have an ambiguous conclusion about this result. The saliva should not be used for HBsAg testing using these devices. The following sentence was included in the conclusion section: “saliva samples should not be used for HBsAg detection with the assays evaluated in the present study”.

- EIA need to be defined in the abstract (enzyme immunoassays). This term was defined in the abstract, as suggested.

- In the abstract: Sensitivity and specificity should be indicated in the abstract section of each test. Conclusion about performances be different should be different in blood versus saliva since the performances is not acceptable in saliva. Values of sensitivity and specificity and conclusions about RT performance using blood versus saliva are now included in the abstract section.

- Line 79, page 4: “are labor-intensive and time-consuming” is not appropriate for EIA assays since these in vitro assays are relatively easy to perform. This information was removed.

- Line 84, page 4, replace: “and do not require complex laboratory infrastructure” by « and do not require laboratory infrastructure” since laboratory infrastructure for EIA assays cannot be considered as complex, even in low income countries. This sentence was modified, as suggested (line 89).

- The sentence line 87, page 4 “patients’ samples are poured over strips containing impregnated antibodies to HBsAg conjugated with colloidal gold” is not exact since some lateral flow tests used other techniques (ex. Vikia : BSA-biotinylated complex coupled to blue-dyed polystyrene microspheres).
This information was removed, and the sentence in the background section was rewritten (lines 92-95).

- Line 115 page 5: need to be modified: “comprising acute, chronic or suspected cases of hepatitis B infections”
This information was modified; see methods, study population section (lines 120-121).

The reference was included in the reference section (number 20).