Author's response to reviews

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

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Author's response to reviews: see over
Ms Tamarisk Bombales
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Thanks for sending me the manuscript reviewed entitled “Evaluating HBsAg rapid tests performance in different biological samples from low- and high infection rates 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, accompanied with the comments of the referees.

We are in agreement with the observations made by the reviewers that has modified and improved our manuscript consequently. These modifications are displayed in the text in red color.

I am sending the answers to your queries, the same is presented in the body of the text.

We send some observations about the reviewer’s comments.

Sincerely yours,

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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 was 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.
  All rapid tests are approved by Brazilian National Health Surveillance Agency (ANVISA). ANVISA is responsible for the regulation, control and supervision of products and services that involve risk to public health.
  Vikia® HBsAg has CE IVD approve but it does not have FDA approval or WHO registration. Imuno-Rápido HBsAg® do not have FDA approval or WHO registration and these information is not available for HBsAg Teste Rápido®.
  These data were included in methods section, Rapid tests evaluation section, lines 189 to 194.

- 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 (BioMerieux, Doles or Wama) and better than the present mix.
  We agree with this observation and the same designation as Vikia HBsAg, HBsAg Teste Rápido or Imuno-Rápido HBsAg was used all along 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 evaluation of rapid tests for HBV. As rapid tests were not developed for saliva samples, sample volume was increased. Indeed, the results obtained were not satisfactory, but this information could be helpful in order to demonstrate that these assays could not be used for 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).
  The references were included in References section (numbers 20 and 21) and were included in Discussion section lines 365-371.
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).

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

   Group I was composed by individuals recruited at Viral Hepatitis Ambulatory who gave 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 the attendance at Fiocruz Viral Hepatitis Ambulatory, to reside in underserved and impoverished areas in the Rio de Janeiro State and being a suspected case of viral hepatitis infection.

   In Group II, individuals from general population of two cities of Rio de Janeiro State were included. Petrópolis city is located in mountain region while Macaé city is situated in North region of Rio de Janeiro State. None of these individuals were recruited in viral hepatitis ambulatory and this group was considered a low risk for HBV. This information was improved in 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 were included in a table (Appendix 2) and presented in results and discussion section.

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.

   In order to confirm HBsAg results, all reactive HBsAg serum samples were retested in duplicate and only concordant results were included in the study. Indeed, AgHBs neutralization assays, and HBV DNA testing, HBsAg concentration were not determined and this limitation was included in discussion section as suggested line 388-390.
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.
Analytical sensitivity was included in methods section, rapid tests evaluations section, lines 200-202. The possibility to use capillary blood was indicated in the text in section Rapid tests evaluation 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 discussion section as suggest in lines 334 to 336.

- 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 conclusion section: “Saliva samples should not be used for HBsAg detection using the devices 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 performances of RT in blood versus saliva were included in 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 excluded.

- 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 in 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 sentence was rewritten in Background section 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 in Methods, study population section lines 120-121.

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