Author's response to reviews

Title: Clinical features and pitfalls in the laboratory diagnosis of dengue in travellers

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Author's response to reviews: see over
Submission of revised manuscript

Dear Editors,

I wish to submit in the name of all authors the revised manuscript entitled “Clinical features and pitfalls in the laboratory diagnosis of dengue in travelers” for consideration for publication as a research article in BMC Infectious Diseases.

According to the editors’ advice, formatting of the text has been performed with the help of the provided MS-word template. Minor changes have been performed by formatting the references (italics for the journals’ names). As suggested by the referees, the method section has been restructured for better understanding. A statement has been included that the research has been conducted in accordance to an ethical committee and in compliance with the Helsinki Declaration (line 15-16 on the first page of the methods section).

The point-by-point response to the referees is included at the end of this letter.

Yours sincerely

Ole Wichmann
MD, MCTM, DTM&H
Point-by-point response to the referees

Referee 1

“The manuscript by Wichmann et al. describes the evaluation of clinical manifestations, haematologic findings and serology results obtained with a commercial ELISA kit to predict the correct diagnosis of dengue infections. Their conclusion that the ELISA kit used in their study has a positive predictive value of only 50% goes against the common knowledge of those who have used the very same kit. Also, their explanation for their findings is not convincing. They defend that, in their patient sample, other infections might have been involved in yielding false-positive results. That is hard to agree to, since in regions where several flavivirus co-circulate, these false-positive results have not been observed, and the sensitivity and specificity have been usually high.”

→ As outlined in the discussion section, we agree to the referee that previous studies have demonstrated good test results with the PanBio kit: “[.....] The combination of these two tests showed excellent sensitivity (99-100%) in populations of endemic countries in Asia [13, 18]. Vaughn et al. demonstrated a high specificity (92%) in paired sera from Thai patients without flavivirus infection […].” However, we designed the study to have a look at the positive predictive value of the PanBio ELISA kits in single serum specimen only. The reason for this we discussed in the introduction: it is very common that a febrile patient presents in the outpatient department, and from a single serum sample the clinician is forced to establish the diagnosis. The patient who feels sick would like to know as soon as possible what the cause of his illness is (and not when presenting a second time to collect a second serum sample!). In addition, the clinician can take decision to rule out other potential diseases by additional (most often expensive!) serological tests on the basis of the interpretation of the single serum sample dengue test result. Furthermore, in literature there are several studies on dengue with a dengue diagnosis established on single serum samples only. With our study we are able to demonstrate that these results need to be interpreted with caution!

This we tried to point out in our conclusion, reinforcing that the interpretation of paired serum samples (also with the PanBio ELISA!) is most reliable and confirming for the serological diagnosis of dengue fever.

Further possible reasons for the false positive PanBio ELISA results are given in the revised manuscript on the third page of the discussion section (line 16-20): “Overall, possible explanations for false positive PanBio-ELISA results are that the cut-off value defined by a calibrator in each batch was too low (and fits better for suspected cases in endemic areas than for travellers of non-endemic areas), cross-reactive flavivirus-specific IgM antibodies, non-specific bindings of IgM antibodies from other infections, and rheumatoid factor.”

“The methods section is fairly written but lacks a more detailed description of the new E/M and NS1 ELISA kits since these kits are not widely used. Also, the results obtained with these kits have not been described on the results section, and appear only in the discussion section.”

→ As recommended by the referee, a more detailed description of the E/M and NS1 ELISA kits has been included on the second page of the method section (line 4-13). The results relevant to this paper are now presented in the revised version in more detail (at the end of the first page of the result section and the beginning of the second page). The serotyping-results of the confirmed infections have been published previously (as cited by reference 14)!
Furthermore, their description of the patients enrolled on the study is confused and they have to redo their math. For example, they say that they enrolled 1,092 patients with travel-associated fever, but when the febrile patients returning from Asia (514), Africa (346), and South/Central America and Caribbean (230) are added together, the result is 1,090. They should review their data, and make a better description of them. The discrepancy in the numbers added results from the fact that three patients travelled to more than one continent (two with fever and one with diarrhoea). These three patients have been excluded from the continent-specific analysis. This explanation has now been included in the revised version (line 9-10 on the first page of the result-section).

However, a major flaw of their work is that they have tested all samples without a clear definition of which day of the disease the sample was collected. It is a well-known fact that if a test is performed on the febrile period, ELISA will be negative. That might explain their negative results. Information about the day of blood collection after onset of symptoms was available in the study. The day of blood collection were classified in days by 3 (day 1-3, day 4-6, day 7-9, etc.). This classification for the whole population was included in the revised version in the result section (line 5-6). Most importantly, figure 2 classifies all PanBio-ELISA-positive samples according to time of collection after disease onset and also demonstrates, that false negative results only occurred on the first three days.

Even though they show on figure 1 that they have performed RT-PCR on these samples, the results are neither shown nor discussed. All sero-negative samples collected < 8 days after onset of symptoms were tested by RT-PCR (results shown on page 2 of the result section, line 6-9). Furthermore, RT-PCR was performed in PanBio-ELISA sero-positive cases only if the E/M and NS1 ELISA results were different from the IFA. These results are mentioned at the end of page 1 of the result section.

Major Compulsory Revisions: Redo the math on their description of sample. Better description of the patients analysed with each diagnostic test shown on figure 1. The discrepancy in the numbers has been explained above and has been added in the revised version. As recommended by the referee, the diagnostic tests have been described in more details in the method section (page two of method section).

Minor Compulsory Revisions: Table 2 is not necessary. As recommended by the referee, table 2 has been deleted with some of the important results added in the text (result section, in the end of the “clinical and laboratory features“)

Referee 2

From the results presented here, it is not easy to clearly and accurately define the positive or negative tested samples although a high number of specimens were tested and several tests employed. Also, it is not easy to follow obtained results. Results: Paragraphs 2 and 3: It is not clear how the infection was confirmed in 64/127 samples with a probable dengue infection. The method section and the result section have been re-structured and more detailed information are given straightforward in the revised version of the manuscript.

The study was conducted in order to point out the problem of interpreting a single serum sample (see also the reply to referee 1, first comment). This is frequently done in clinical practise, and there are also several published studies on dengue using single serum samples only (due to the lack of paired samples which are required—for example by the WHO- to confirm the diagnosis).
As outlined now more clearly in the method section, we first performed a screening test with PanBio IgM and IgG ELISAs. Since this study was conducted retrospectively, only single serum samples were available. Thus, for confirmation we have further tested every PanBio-ELISA positive sample with four assays: E/M and NS 1 serotype-specific capture IgM-ELISAs, a NS1 serotype-specific IgG ELISA, and IFT. By definition, a PanBio-ELISA screening test result has been classified as “true positive” if the combination of these four tests was positive. If results of these four tests were discrepant, RT-PCR was applied if sera were collected in the acute phase of disease. Especially the NS1 isotype- and serotype-specific IgM and IgG-ELISAs have demonstrated good correlation with dengue virus PRNT, which is the “gold standard” for serological analysis.

" The 10 positive samples defined by high IgG antibody and the 4 with both IgG and IgM antibodies how were classified? These samples are included into the final 64 "confirmed" samples????

→ Of the 10 positive samples defined by high IgG only, 5 turned out to be true positive, 4 turned out to be false positive, and one was not interpretable. This is now more clearly stated in the result section of the revised manuscript under “details of serological results”.

Of the 4 samples with both positive IgG and IgM antibodies in the PanBio ELISA, all were classified as true positive in accordance with all four confirmatory tests (two interpreted as primary and two as secondary infections).

"How it is possible to guarantee that negative samples are really negatives? Frequently, RT/PCR is negative and seroconversion is observed."

→ We agree with this comment. We included this point in the discussion section (top of second page of the discussion). However, the major aim of this article was to point out the problem of false positive dengue serology in single serum samples, and how to improve the predictive value of those tests if performed in single serum samples.

"Introduction: could be shortened"

→ As recommended by the referee, the introduction has been shortened by deleting several sentences (in the old version: line 13-16 on the first page of the introduction; line 3, 11, and 12 on the second page of the introduction).

"Materials and methods: samples should be classified according the time of collection after disease onset"

→ The day of blood collection were classified in days by 3 (day 1-3, day 4-6, day 7-9, etc.). This classification for the whole population was included in the revised version in the result section (line 4-6). Most importantly, figure 2 classifies all PanBio-ELISA-positive samples according to time of collection after disease onset and also demonstrates that false negative serological results only occurred on the first three days.

"Results: Paragraph 1: Verify figures of origin of patients"

→ The discrepancy in the numbers results from the fact that three patients travelled to more than one continent (two with fever and one with diarrhoea). These three patients have been excluded from the continent-specific analysis. This explanation has now been included of the revised version (line 8-9 on the first page of the result-section).